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A NEW ORGANIC MERCURY COMPOUND WITH POWERFUL GERMICIDAL PROPERTIES*

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ONE of us, in collaboration with Drs. J. F. Schamberg and J. A. Kolmer, has previously found that the introduction of a nitro group into organic compounds of mercury greatly enhances the bactericidal properties of the latter. One of such compounds, sodium hydroxymercuri-ortho-nitro-phenolate, known under the name of Mercurophen, has been described[1] and recommended as a local antiseptic. Subsequently, it has been found by Charles H. de T. Shivers to be a valuable aid in the treatment of gonorrhea[2], while others utilized the compound in disinfection of suppurative wounds, in the treatment of pneumococcus conjunctivitis, chronic pneumococcic ulceration of the cornea and various pyogenic skin conditions.

Thus it appears that the nitro group, which has been pronounced by Ehrlich[3] and subsequently found by us in our own work, to be dystherapeutic —or at least greatly inferior to the amino group—this same group becomes eutherapeutic and much superior to the amino radical, when introduced into the benzene ring of an organic mercurial. The former seems to increase the affinity of mercury for pathogenic microorganisms, particularly *in vitro*.

It is possible that this increase in the parasiticidal effect of such mercury derivatives is due to their unusual stability both in powder form and in aqueous solution, which is not the case with the compounds containing amino groups. The sodium salts of the latter class of compounds have been found by one of us in conjunction with A. Proskouriakoff[4] to be generally unstable in aqueous solutions, the mercury splitting off from the molecule, and depositing as a fine gray powder.

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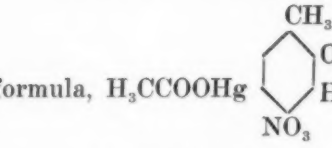
It was with the view of further study of these nitro compounds that the various mononitrocresols have been investigated. Ten such nitrocresols are theoretically possible. Of these one, 4-nitro-2-cresol, deserves particular attention as its mercuration gave us our preparation No. 381, a new powerful mercurial germicide. For the sake of convenience we call it "Metaphen," its chemical name being 4-nitro-3, 5-bisacetoxymercuri-2-cresol, and its structural formula,  It is a heavy brownish-yellow powder insoluble in water, but completely soluble in dilute aqueous sodium hydroxide, the color of its solutions being red in high concentrations and amber in lower. In concentrations sufficient to exert germicidal effect, these solutions have no staining properties on either human tissue or linen.

Its toxicity has been tested on white rats, which, when injected by the intravenous route, easily tolerated doses of 4 mg. per kilo. Intramuscularly, however, metaphen has been tolerated by rats without or with slight pain in doses of 30 mg., and even in doses of 50 mg. per kilo, though in the latter cases the animals experienced much pain at the site of injection during the administration of the drug.

In order to determine the germicidal power of the new compound, we used principally two methods:

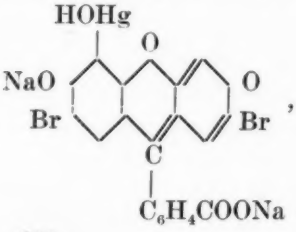
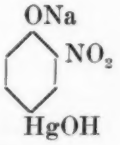
A. Exposure of the bacteria to the chemical for one hour. This was carried out as follows: Solutions of the chemicals in sterile water were made up in varied concentrations, and 4 c.c. of each dilution were placed in each of a series of sterile test tubes. The test organisms used (Staphylococcus aureus, Bacillus typhosus) were grown in broth for 24 hours, shaken with beads to break up clumps, diluted with sterile physiologic salt solution until a definite suspension was obtained by comparing it with the standard adopted in our laboratories, and 1 c.c. added to each test tube, making the total volume 5 c.c. In this way we were sure that each tube was seeded with an approximately equal number of bacteria. Owing to the simplicity of the technic, the possible errors were reduced to a minimum. In each experiment controls of the cultures and sterile salt solution were included.

After the culture has been added to the solution of the chemical, the test tubes were incubated for one hour at 37°C., and subcultures made of each test tube on agar slants by means of a 4 mm. platinum loop. These subcultures were observed at the end of 24 and 48 hours.

B. Exposure for 4 days. In this series of experiments only 1 c.c. of each dilution of the chemical was placed in each test tube. The test organism was grown in broth for 24 hours, shaken with beads to break up clumps, and 1 c.c. added to 100 c.c. of broth of which 5 c.c. were added to each test tube, making the total volume 6 c.c. The test tubes were then incubated for four days at 37°C., and observed every 24 hours. The tubes were con-

sidered sterile when they looked perfectly clear, but to determine whether complete destruction of all the bacteria took place, subcultures on agar slants were made at the expiration of the incubation period. In each experiment controls of the cultures and sterile salt solution were included.

We are aware of the limitations attached to both of these methods. The removal of a loopful of culture for the purpose of transplanting on agar slants must invariably be accompanied by error, since minute quantities of the chemical are thus transferred to the agar, continuing its destructive action upon the bacteria. Furthermore, the virulence of bacteria and their resistance to chemicals is subject to great variation, so that no two experiments yield the same results.

In order to be able to draw conclusions from work of this kind, they must be interpreted in a strictly comparative sense. The value of a new chemical as a germicide can be arrived at only when compared with some well known compounds. Accordingly, we included in our study not only bichloride of mercury, the action of which is generally known, but also such known germicides as mercurochrome,  , mercurosal, $C_6H_3 \begin{cases} COONa \\ OCH_2COONa \\ HgOH \end{cases}$, and mercurophen,  . All these chemicals have been investigated under the same conditions as metaphen or No. 381, to permit strictly comparative conclusions. The results are shown in Tables I to IV.

Method A. Exposure for One Hour

Table I—Staphylococcus Aureus

Substance	% of Hg.	Bactericidal Dilution*	$HgCl_2$ Index**	M.T.D.†	Germicidal Index‡
Mercuric chloride	73.89	1:106,000	1.00	2	2.00
Mercurophen	53	1:81,000	0.76	6	4.56
Metaphen	59.98	1:170,000	1.60	4	6.40
Mercurochrome	26	1:4,500	0.042	32	1.34
Mercurosal	43.5	1:3 000	0.028	20	0.56

Table II—Bacillus Typhosus

Mercuric chloride	73.89	1:124,000	1.00	2	2.00
Mecurophen	53	1:94,000	0.76	6	4.56
Metaphen	59.98	1:134,000	1.08	4	4.32
Mercurochrome	26	1:3,000	0.024	32	0.77
Mercurosal	43.5	1:4,000	0.032	20	0.64

*Average of 4 to 6 experiments.

**The bichloride of mercury index is obtained by considering the germicidal power of mercuric chloride as unity, and computing the corresponding values of the other chemicals.

†M.T.D. is the maximum dose (mg. per kilo. of body weight) tolerated by white rats when injected intravenously.

‡The germicidal index is the product obtained by multiplying the bichloride of mercury index by the M.T.D.

Method B. Exposure for Four Days

Table III—Staphylococcus Aureus

Substance	Dilutions inhibiting growth after*				Bactericidal* dilution at end of 4 days	Bicholoride of mercury index	Germicidal index
	24 hrs.	48 hrs.	72 hrs.	96 hrs.			
Mercuric chloride	1:960,000	1:480,000	1:396,000	1:372,000	1:336,000	1.00	2.00
Mercurophen	1:20,400,000	1:3,840,000	1:2,760,000	1:2,520,000	1:2,400,000	7.14	42.84
Metaphen	1:20,400,000	1:7,560,000	1:5,760,000	1:4,080,000	1:3,850,000	11.46	45.74
Mercurochrome	1:73,500	1:45,000	1:42,000	1:42,000	1:36,000	0.11	3.52
Mercurosal	1:19,500	1:18,500	1:18,500	1:18,500	1:18,000	0.05	1.00

Table IV—Bacillus Typhosus

Mercuric chloride	1:600,000	1:456,000	1:408,000	1:324,000	1:228,000	1.00	2.00
Mercurophen	1:252,000	1:84,000	1:72,000	1:60,000	1:56,500	0.25	1.50
Metaphen	1:552,000	1:276,000	1:252,000	1:252,000	1:252,000	1.11	4.44
Mercurochrome	1:75,000	1:33,000	1:30,000	1:28,500	1:21,000	0.092	2.94
Mercurosal	1:39,000	1:10,500	1:10,500	1:7,500	1:7,500	0.033	0.66

*Average of 4 to 6 experiments.

A glance at Tables I and II will at once reveal the superiority of metaphen over the other chemicals with which it has been compared. Its germicidal power for Staphylococcus aureus is more than one and one-half times as great as that of bichloride of mercury, which is also inferior to the new mercurial in its effect on Bacillus typhosus, and were we to make the comparison on the basis of percentage of pure mercury contained in the compounds, the superiority of metaphen would be shown to be still greater.

When we come to study Tables III and IV we are impressed with the strikingly high destructive effect of both metaphen and mercurophen upon Staphylococcus aureus after exposure for 24 or more hours. The specific action of organic mercurials containing nitro groups upon staphylococci is quite evident here. At the end of 24 hours the inhibitory power of metaphen against Staphylococcus aureus is more than twenty times that of mercuric chloride, and its germicidal effect after four days' exposure eleven times as great. For Bacillus typhosus metaphen is not so destructive after long exposure, but its superiority over the other organic mercurials even against this microorganism is maintained throughout.

Another important point to consider is the germicidal index. This has already been defined as the product of the bichloride of mercury index and the M.T.D. For it is evident that from a chemotherapeutic standpoint the bactericidal value alone is not sufficient. We must also know the toxicity of the particular compound in order to get a fair idea of its value as a chemotherapeutic agent. And here again the value for metaphen is more than three times as great as that of bichloride of mercury.

We realize, however, that the germicidal index is only one factor in the determination of the usefulness of a new therapeutic agent. There are other properties which must be taken into account, such as the effect of the presence of serum proteins which frequently lower the germicidal activity of the chemical, because of the latter's greater affinity for the proteins of the body than for the bacteria. Other facts to be considered are the stability of the drug, its penetrating power, its influence on the phagocytic activity of polymorphonuclear leucocytes, and its irritability to the skin, mucous membranes etc.

With regard to the serum proteins our experiments have shown that these do not readily combine with the compound under discussion.

Table V clearly indicates how reluctant metaphen is to interact proteins. A concentration of even 1:200 shows no trace of a precipitate, whereas in

TABLE V

SUBSTANCE	FINAL DILUTION	1:200	1:300	1:400	1:600	1:800	1:1000	1:1200	1:1600
Mercuric chloride	Serum 50%	H. P.	H. P.	H. P.	H. P.	M. P.	S. P.	S. P.	–
Metaphen (#381)	Serum 50%	–	–	–	–	–	–	–	–

H. P.=Heavy precipitate. M. P.=Moderate precipitate. S. P. = Slight precipitate. – = No precipitate.

the case of mercuric chloride a slight precipitate is already noticeable in concentrations as low as 1:1200.

This nonprecipitating effect of metaphen upon serum proteins may probably account for the fact that it is but very slightly irritating to the skin and mucous membranes in therapeutic dilutions. Thus, three instillations into the eye of a normal rabbit of two drops of metaphen in a dilution of 1:100 resulted only in a mild hyperemia of the conjunctiva, while similar instillations of mercuric chloride solution of the same concentrations resulted in severe purulent conjunctivitis.

In another experiment 2 drops of a 1:1000 solution of metaphen were instilled into the right eye of a normal rabbit daily for eleven days, without any deleterious effects. From the twelfth day up to and including the twentieth a solution of 1:100 was similarly employed, without any untoward effects, other than a very slight amount of pus on the twelfth day. The condition of the left eye, which was similarly treated with a 1:1000 solution of mercuric chloride, became so threatening on the fourth day, that further instillations had to be abandoned.

Similar experiments have been conducted with the nose and throat of rabbits. Into the former daily instillations of 2 drops of a 1:10,000 solution of metaphen for eleven days, and subsequently 2 drops of a 1:100 solution for nine more days, showed no harmful effects whatsoever, while similar results were obtained upon instilling into the throat of the rabbit 0.5 c.c. of a 1:10,000 solution of metaphen daily for eleven days, and subsequently similar quantities of a 1:100 solution for nine more days.

We were also interested to find out the effect of metaphen when administered per os to rabbits. Two animals were selected for this purpose. To the first 10 c.c. of a 1:1000 solution was given by a stomach tube, and 14 days later another 10 c.c. of a 1:100 solution, while to the second 10 c.c. of a 1:300 solution was administered by the same route. In neither case were any reactions observed, either at the time of the administration of the drug or afterwards. No albumin was found in the urine. However, both animals gradually lost weight for a period of about ten days after the administration of the chemical, after which their weight slowly began to increase.

A fact about metaphen that should not be overlooked is the non-tarnishing effect of its bactericidal solutions upon various metallic instruments which, when immersed in a 1:1000 solution of the compound for one week, show no change in their appearance. Bichloride of mercury in the same concentration tarnishes instruments within 24 hours.

Metaphen also possesses powerful spirocheticidal properties, which is evidenced by its effect in the treatment of rabbits infected with Treponema pallidum according to the method of Brown and Pearce.[5] The results are shown in Tables VI and VII.

These tables show distinctly the favorable effect of metaphen in experimental syphilis. In rabbit 19 a single intravenous injection cleared the blood of spirochetes within 48 hours, and although a few reappeared on the follow-

ing day, they disappeared permanently six days later. Clinically both testicles became normal on the forty-second day after injection and remained so until the last day of our observation—nearly three months.

However, in another animal this route of treatment did not yield such good results. Rabbit 66, whose complete history we omit on account of the lack of space, was heavily infected, having a large syphiloma with a considerable sized chancre of the right testicle. An intravenous injection of 2 mg. per kilo of metaphen resulted in the sterilization of the blood within 5 days and the healing of the chancre about 3 weeks later. But on the thirtieth day a relapse occurred, and intramuscular treatment was begun on the forty-seventh day. Nine such injections of 1 mg. per kilo were given during the next 33 days, the blood becoming permanently free of spirochetes on the twenty-eighth day and testicles normal on the thirty-third. The animal appeared to be normal on the last day of our observation, 74 days after intramuscular treatment had been begun.

The efficacy of the intramuscular route is also evident in the case of rabbit 65, which has been cured within 15 days, after only five injections given at intervals of 3 to 4 days. The animal remained cured till the last day of our observation, i. e., for 83 days. This method is to be preferred because in this way only $\frac{1}{50}$ to $\frac{1}{25}$ of the maximum tolerated dose is sufficient for a single injection, thus leaving a wide margin of safety.

Moreover, no indurations occurred as a rule, and those that did occur were slight. The injections of the doses indicated have been painless, as the animals remained quiet during the injections, and their ability to walk was not impaired in any way.

Finally, we may mention that although no direct experiments on the action of metaphen on gonococci have been performed, the solution of the compound (1:5000) has been employed in the treatment of gonorrheal urethritis with very good results, the urethral discharge stopping in the majority of cases, after one or two treatments.

CONCLUSIONS

4-Nitro-3, 5-bisacetoxymercuri-2-cresol, for convenience designated by us as "Metaphen," possesses certain superior properties, as follows:

1. It is a very powerful germicidal substance, particularly against staphylococci.

2. It has a comparatively high bichloride of mercury index.

3. It does not precipitate proteins in as high concentration as 1:200, while the maximum concentration for all practical purposes should not exceed 1:1000.

4. It practically does not irritate the skin or mucous membranes; nor does it tarnish surgical instruments.

5. It has a low toxicity when administered intramuscularly.

6. It possesses powerful spirocheticidal properties.

7. Because of its high destructive effect upon bacilli, and its many favorable biologic properties, it should find practical applications in therapy.

TABLE VI

RABBIT 19*

WEIGHT IN GM.	DOSE IN GM. PER KILO	ROUTE	DAYS AFTER INJECTION	LEFT TESTICLE		RIGHT TESTICLE	
				CLINICAL OBSERVATIONS	DARK-FIELD EXAMINATION FOR SPIROCHETES	CLINICAL OBSERVATIONS	DARK-FIELD EXAMINATION FOR SPIROCHETES
1875	0.002	Intravenous	1	Medium very hard node	++++ Quite motile	Medium very hard node	+ Quite motile
			2	Same	Negative	Same	Few
			3	"	"	"	Negative
			6		Few Quite motile		Few. Quite motile
			9	Node smaller	Few	Node smaller	
			13	Smaller and softer	Negative	Smaller and softer	Negative
			20	Very small soft node	"	Very small soft node	"
			29	Small node	"	Small node	"
			36	Very small node	"	Very small node	"
			42	Same	"	Same	"
			52	Normal	"	Normal	"
			79	"	"	"	"
			104	Small node	"	"	"
			125	Normal	"	"	"
				"	"	"	"

*This rabbit had been unsuccessfully treated with other chemicals for three months before the injection of metaphen had been given.

TABLE VII

RABBIT 65, INOCULATED 50 DAYS BEFORE TREATMENT

WEIGHT IN GM.	DOSE IN GM. PER KILO	ROUTE	DAYS AFTER INJECTION	LEFT TESTICLE		RIGHT TESTICLE		REMARKS
				CLINICAL OBSERVATIONS	DARK-FIELD EXAMINATION FOR SPIROCHETES	CLINICAL OBSERVATIONS	DARK-FIELD EXAMINATION FOR SPIROCHETES	
2310	0.001 in 2 c.c. H_2O	Intramuscular (leg)		Enlarged. Small node. Slight edema	Negative	Enlarged. Small hard node. Slight edema	+++ Very active	++++ = Very numerous +++ = numerous ++ = 4–6 in each field + = 2–3 in each field
2210			1	Very small node	Negative	Small node	Negative	No induration at site of injection
2250			2	" " "	"	" "	"	"
2300	0.001 in 2 c.c. H_2O		4	Small node	"	Medium node	+ Quite motile	
2230			5	Slight edema. Very small node	"	Slight edema Small node	Few. Quite motile	Small induration, no pain
2310	0.001		8	Small node	"	Small node	Negative	
2500	0.001		12	About normal	"	About normal	"	
2300	0.001		15	Normal	"	Normal	"	No induration followed
			98	"		"		

We would like to acknowledge our indebtedness for valuable cooperation in this work to J. S. Stigloos and W. R. Winicov, members of these laboratories.

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[2]Jour. Urol., 1922, viii, 35.
[3]Die Chemotherapie der spirillosen, Berlin, 1910, p. 124.
[4]Jour. Am. Chem. Soc., 1922, xliv, 788.
[5]Jour. Exper. Med., 1920, xxxi, 475.

SUPPOSED DISTURBING FACTORS IN THE WASSERMANN REACTION*

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THOUGH the Wassermann reaction has been criticized as nonspecific, it remains, up to the present, the only reaction of approved reliability in the diagnosis of syphilis, provided frambesia tropica and leprosy (*forma tuberosa*) can be excluded, in which diseases it also gives a positive reaction.

There are some other pathologic conditions in which with the Wassermann reaction positive results have been, from time to time, recorded, e.g., psoriasis, malaria, pellagra, tuberculosis, pneumonia, scarlet fever, and fever in general. With other observers, I am of the opinion, that these, however, are false interpretations, due in all likelihood to technical faults in the carrying out of the test. With scrupulous attention to technic there is no doubt that the Wassermann reaction in such cases invariably gives negative results.

There remain one or two other conditions in which anomalous results have been obtained with this reaction. Though I have failed to find any accounts of detailed work on the subject, in some textbooks of pathology and of biochemistry it is asserted that chloroform and ether anesthesia, likewise the presence of fat in the serum after ingestion of a fatty meal, may give rise to results which simulate a luetic infection. So long as such exceptional cases stand on record we are obviously unable to claim the Wassermann reaction as exclusively pathognomonic of luetic infection, and it is of importance that the observations should be controlled with a view either to confirmation or the reverse. The object of this present research has been to investigate these anomalous cases.

TECHNIC

The technic employed in the investigation was that in use in Wassermann's laboratory in 1910, with the addition of certain modifications which since then have received general approval. On the whole, the method of

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Harrison[1] has been adhered to, except that the hand method was preferred to the dropping method and in every case 0.5 c.c. was used, bringing the total volume to 2.5 c.c. So far as possible, all titrations and the preparation of material were carried out under my own supervision in the laboratory.

The following notes apply to the reagents used:

1. *Amboceptor Against Sheep's Erythrocytes.*—This was prepared in the physiology department. The titre was between 1:3000 and 1:5000. Amboceptors with a titre inferior to 1:1000 were discarded; 4 M.H.D. were used.

2. *Sheep's Blood.*—This was obtained from the local abattoir as nearly sterile as possible. The corpuscles were washed four times with a constant number of revolutions, and in each case for a similar period of time.

3. *Complement.*—This consisted of the fresh serum of two guinea pigs, sacrificed on the night previous to the experiment. 2 M.H.D. of complement was used after titration by itself and with antigen.

4. *Extracts.*—In every case three different extracts were used in one and the same experiment, viz.: (a) a commercial guinea pig heart extract with cholesterol (Burroughs, Wellcome & Co.); (b) a guinea pig heart extract prepared according to the method of Fildes and McIntosh,[2] to which at the time of use a 1:100 alcoholic solution of cholesterol was added; (c) acetone insoluble, alcohol soluble, calf's heart extract, prepared according to the method of Bordet and Ruelens,[3] to which at the time of use a 1 per cent alcoholic solution of cholesterol was added. The extracts were repeatedly titrated beforehand with three rows of test tubes, one containing luetic serum, the second normal serum, the third saline solution. For the titration several different luetic and normal sera were used.

5. *Sera.*—The sera to be tested were obtained from animals by the method of heart puncture, the blood being received through aseptic apparatus into sterile test tubes. The clear serum, expressed after coagulation of the blood was inactivated for thirty minutes at 55° C., and then kept in the ice chest in readiness for the test, being warmed to 55° C. just before the test. The saline solution, 0.9 per cent NaCl, was sterilized in the autoclave under 20 pounds pressure. The quantity of each substance used was brought up to 0.5 c.c. with NaCl solution.

In the main test, before incubating for half an hour, previous to the addition of the sensitized sheep's cells, I let the sera, with extract and complement, stand at room temperature (16 to 20° C.) for half an hour.

All glassware used was cleansed with a hot solution of sulphuric acid and bichromate, then with sodium bicarbonate solution, followed by ordinary tap water and distilled water. After drying it was sterilized in the usual way by enclosure for thirty minutes in an oven at 120° C.

I. DOES CHLOROFORM OR ETHER INFLUENCE THE WASSERMANN REACTION?

Chloroform, ether, and other narcotics are said to produce an increased amount of free lipins in the blood, and the assumption is that these lipins in a nonsyphilitic serum account for the positive Wassermann reaction. In this connection, two lines of investigation were employed: (1) the effect of

chloroform and ether added directly to the blood *in vitro;* (2) the effect of general anesthesia with chloroform and with ether.

The former question may be dealt with first. Specimens of defibrinated blood (of sheep, of cats and of dogs) were collected in large sterile test tubes and connected with a flask containing chloroform or ether, as the case might be, through which flask a current of air was made to pass by means of an electric pump. In this way the anesthetic vapor came in direct contact with the blood, which, at regular intervals, was thoroughly shaken. It was later found that if a test tube containing ether or chloroform was placed inside a stoppered Erlenmeyer flask, the bottom portion of which contained the defibrinated blood, hemolysis rapidly occurred, *pari passu* with absorption by the blood of the volatile anesthetic. By this latter device all splashing and frothing are avoided, interaction between the blood and the anesthetic vapor taking place silently and quietly. After a certain time, which varied from ten minutes to one hour, the blood was centrifuged. The clear serum, red owing to the presence of dissolved hemoglobin, was pipetted into a sterile test tube, and inactivated for thirty minutes at 55° C.; in this process the serum, if containing a large amount of hemoglobin, acquired a dark chocolate color. It was then tested by the Wassermann reaction.

The result of application of the anesthetic directly to the blood can be stated in a clear and categorical fashion. Thirty samples of blood (ten of dog, ten of cat, and ten of sheep) were treated with chloroform. In no case did the slightest sign of a positive reaction appear. An identical statement, word for word, applies to the investigation with ether, and to the results therewith obtained. Should any one desire to repeat this experiment, it is only fair to point out that occasionally, and particularly when the amount of hemolysis, through the long delayed action of the chloroform or of the ether, is large, the serum gives an *anticomplementary* reaction, which, however, can be readily distinguished from a positive Wassermann result.

The second series of experiments, which from the practical point of view is of most importance, was carried out on a large number of animals, mostly dogs and cats. The animals, having received no food for at least twelve hours previously, were in some cases injected subcutaneously with 0.5 c.c. of a 2 per cent solution of morphine per kilo, one hour before being anesthetized; in other cases they were put under anesthesia without any morphine. The animals were kept under complete anesthesia for two and a half hours, and the blood, 5 to 10 c.c. at a time, was drawn by heart puncture every fifteen minutes, with aseptic precautions, from the circulation. In cats and in guinea pigs the amount of blood thus obtained was naturally less than in dogs. Specimens of blood from the same animal were in this way secured at different intervals. The serum was prepared in the usual way, and then inactivated for thirty minutes at 55° C.

As in the previous set of experiments, the results in this series were uniformly negative. Once or twice the same serum tested with three different extracts gave a doubtful result (– +) with one of the antigens. As the doubtful reading occurred in each case with the same extract, and as this

extract by itself was found to give a trace of inhibition of hemolysis, it was considered legitimate and in accordance with recognized canons to interpret the result as negative.

II. DRUGS OTHER THAN GENERAL ANESTHETICS

Owing to the provision of a mammalian operative class for students in this department, it was possible to carry out experiments on an unusually large number of animals. Furthermore, it was possible to test the blood of anesthetized animals after injection of various substances such as morphine, atropine, caffeine, amyl nitrite, adrenaline, pituitary extract and secretine. In these cases the blood was obtained either directly from the heart or from the carotid.

As before, the results were wholly and entirely negative. It is of some slight interest to know that the injection of these drugs does not affect the result of the Wassermann reaction. In particular, morphine[4] is a narcotic which might conceivably be considered as exercising an effect on blood similar to that of ether, viz., of increasing the amount of circulating lipins.

III. FAT DIGESTION

It is well known that the blood normally contains only a small percentage of fat. After a fatty meal, however, it may contain so large an amount that the fat actually rises to the surface of the serum like cream.[5] "In the dog the percentage of fat in the blood is remarkably constant under normal conditions. After a fatty meal the increase of fat begins in about an hour and reaches its maximum in about six."[6] A series of sera collected from fasting dogs was compared with other series of sera of the same animals obtained at different periods after a fatty meal. The two sets of sera showed macroscopically a very striking difference. While the sera of the fasting animals were absolutely clear, those taken during digestion showed

TABLE I

RESULTS OF THE WASSERMANN REACTION ON SERA OBTAINED AFTER FATTY MEALS PREVIOUS TO SEPARATION OF FAT

TEST TUBE NO.	SERUM .1	I GUINEA PIG HEART AND CHOLESTEROL	II GUINEA PIG HEART AND CHOLESTEROL (B. & W.)	III CALF HEART AND CHOLESTEROL	IV NaCl SOLUTION
1	Ser. 24	++	++	+	+
2	" 26	+	+	−+	+
3	" 28	−+	+	−+	+
4	" 30	+	+	+	+
5	" 32	++	++	++	++
6	" 34	+	+	+	+
7	" 37	+	++	+	+
8	" 40	+	+	+	+
9	" 42	−+	−+	−+	+
10	" 49	−+	+	+	+
11	" 51	−+	−+	+	+
12	U. S. S. 14	+++	+++	++	−
13	N. S. 19	−	−	−	−
14	NaCl—Ext.	−	−	−	−

a turbid and milky appearance; after standing, the fat rose in a layer to the surface. All the sera were inactivated for thirty minutes at 55° C. The Wassermann reaction was tested both immediately after inactivation, when the globules of fat were still equally distributed throughout the serum, and at a later stage when, after prolonged sojourn of the serum in the ice chest, the fat had risen to the surface leaving the serum clear.

The results in this case are best exhibited by means of Table I, which applies to sera containing naturally admixed fat.

The sera marked 1 to 11 were taken from dogs during fat digestion; No. 12 is an undoubted syphilitic serum from a hospital patient; No. 13 is again a serum from a nonsyphilitic patient taken during fasting; No. 14 is a simple mixture of NaCl solution and extract. As a control all the sera were tested against a 0.9 per cent solution of NaCl, as shown in column IV. It will be noted that all the fat-containing sera gave virtually the same results with the NaCl solution as with the various extracts; in other words, all these sera show an anticomplementary action. We are thus forced to the conclusion that the asserted positivity of fats contained in serum is no true Wassermann reaction.

That the anticomplementary action in question is due to the fat, can be proved by testing the serum after mechanical removal of the fat. Table II shows the results of such a control set of experiments in which the fat-denuded serum is seen to give uniformly negative results.

TABLE II

RESULTS OF WASSERMANN REACTION ON SAME SERA AS IN TABLE I, AFTER SEPARATION OF THE FAT

		I	II	III	IV
TEST TUBE NO.	SERUM .1	GUINEA PIG HEART AND CHOLESTEROL	GUINEA PIG HEART AND CHOLESTEROL (B. & W.)	CALF HEART AND CHOLESTEROL	NaCl SOLUTION
1	Ser. 24	–	–	–	–
2	" 26	–	–	–	–
3	" 28	–	–	–	–
4	" 30	–	–	–	–
5	" 32	–	–	–	–
6	" 34	–	–	–	–
7	" 37	–	–	–	–
8	" 40	–	–	–	–
9	" 42	–	–	–	–
10	" 49	–	–	–	–
11	" 51	–	–	–	–

IV. FEVER

Fever is another factor which in treatises on pathology and on immunology is stated to disturb the regular outcome of the Wassermann reaction, causing an inhibition of hemolysis and thus masking a syphilitic infection.

In its bearing on the Wassermann test a decision on this question is of the same practical importance as a decision on the influence of anesthesia or of the recent ingestion of a meal. Official instructions issued from provincial and from municipal laboratories for the guidance of practitioners tak-

ing samples of blood for the test, frequently warn against taking the blood "after alcohol or general anesthesia," "after a meal," or "if the patient has a high temperature."

Two methods were adopted to study this question. In one case whole blood from man and from animals was heated *in vitro* before the application of the test; in the other case bloods from patients with high fever were subjected to the Wassermann test.

a. *Heated Blood.*—Thirty c.c. of fresh defibrinated blood each of man, calf, sheep, dog and cat were in each case equally distributed in three test tubes, so that each test tube contained 10 c.c. of blood. The fifteen test tubes were put in a water-bath at 60° C. (about 140° F.). After thirty minutes one test tube of each specimen was taken out, after sixty minutes a second specimen of each was removed, while the remainder were removed after two hours. They were all centrifuged at room temperature and the clear serum collected in sterile test tubes. All the sera were then in routine fashion inactivated at 55° C. for thirty minutes, then placed in the ice chest for an hour and finally, on the same day, tested by the Wassermann reaction. The results were uniformly negative.

b. *Sera of Fever Patients.*—By arrangements with hospital authorities in Montreal, I was privileged to obtain blood from a number of patients with high temperatures. The cases selected for examination were of heterogeneous nature and included pneumonias, septicemias, surgical infections, etc., only such cases being selected as had no history of syphilitic infection. The temperature at the time of taking the blood specimen ranged between 101° and 105° F. These results again were wholly negative.

V. CONTROL BY MEANS OF THE Σ REACTION

Lately Dreyer and Ward[7] have proposed "A Simple Quantitative Serum Reaction for the Diagnosis of Syphilis," which, for shortness, they call the "Σ reaction." This reaction, similar in principle to that of the Sachs and Georgi[8] reaction, is based on flocculation produced by the serum of a luetic individual when such serum is put in contact with an acetone insoluble, alcohol soluble extract as prepared by Bordet and Ruelens.

I have for some time been engaged in testing this reaction, not quantitatively but qualitatively, on a large number of luetic and normal sera (see the next succeeding paper of the series).

I have carried out the Σ reaction on a considerable number of sera of animals subjected to chloroform and ether anesthesia; I have made parallel series of observations with the Wassermann and with the Σ reaction on sera of animals obtained during fat digestion, and have made corresponding parallel observations on sera of animals injected with laboratory drugs, and on sera of fever patients; indeed, almost all the sera used in this research were controlled by the Σ reaction. The two sets of investigations were carried out quite independently, comparison of results being made only after the full tests with any given serum were completed. The fact that the sera gave identical results with both reactions seems to me to provide independent

confirmative evidence of what I stated before, viz.: that if a serum does not contain syphilitic "reagins," then chloroform, ether, fat digestion, or fever are powerless to convert an otherwise negative into a positive reaction.

CONCLUSIONS

1. Chloroform and ether, whether added directly to a mammalian, including human blood, or taken up by the blood in the process of anesthesia, are absolutely without influence upon the Wassermann reaction.

2. Sera removed from animals during fat digestion, and therefore containing large quantities of fat, disturb the results of the Wassermann reaction in the direction of simulating a positive reaction. Such apparently positive results are not, however, really so, the influence of the fat being merely anticomplementary; for, the serum by itself, without the extract, gives the same seemingly positive results. If the fat is removed from the serum previously to the test, or if the clear serum is pipetted from the bottom of the test tube after the fat has floated to the surface, the serum gives a wholly negative reaction.

3. The following drugs have been tried as to their possible influence on the Wassermann reaction: Morphine, atropine, caffeine, amyl nitrite, adrenaline, pituitary extract and secretine. They gave negative results.

4. Blood heated *in vitro* to 60° C. and blood from fever patients gave a negative result.

5. All results of 1, 2, 3 and 4 above have been independently controlled by the Σ reaction of Dreyer and Ward and with identical findings.

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THE SODIUM AND POTASSIUM CONTENT OF BLOOD SERUM IN VARIOUS DISEASES AND OF THE CEREBROSPINAL FLUID IN PROVED AND SUSPECTED SYPHILIS*

By Charles S. McVicar, M.D., and Helen Ross, B.A., Rochester, Minn.

WE present here the results of the quantitative determination of sodium and potassium in the blood serum of patients suffering from a variety of diseases, as well as the quantity of these elements found in the cerebrospinal fluid of a series of patients known, or suspected to have syphilis. The study of cerebrospinal fluid was made after we had satisfactorily recovered known amounts of these elements added to the fluid.

In the determination of sodium we followed precisely the original method of Kramer and Tisdall except that tin dishes were sometimes used; we agree with Wilson that tin dishes may be satisfactorily substituted for platinum. It was seldom possible to use more than the slightest suction on the Gooch crucibles; some of the precipitate was easily drawn through, and thus low readings were obtained. Even with scrupulous care we found the method

Table I

Serum in Nephritis

Cases	Diagnosis	Age	Sex	Weight, Pounds	Blood Pressure	Phenolsulphonephthalein Excretion in Two Hours	Blood Urea, Mg. for Each 100 c.c.	Fundus of the Eye	Sodium, Mg. for Each 100 c.c.	Potassium, Mg. for Each 100 c.c.
A297046	Nephritis, duodenal ulcer, appendicitis	52	M	157	124 98	10	214	Negative	382 400	22.4 23.0
A375905	Chronic glomerular nephritis; hypertension; arteriosclerosis	56	M	145	218 130	15	187	Retinitis of arteriosclerosis plus chronic nephritis	187 165	20.2 21.4
A375331	Nephritis; hypertension; arteriosclerosis	25	M	112	194 140	65	27	Chorioretinitis	540 559	21.3 20.3
A379783	Nephritis; hypertension; arteriosclerosis; cardiac decompensation	60	M	148	202 138	40	40	Arteriovenous compression	353 365	21.5 21.4
A374848	Chronic diffuse nephritis; hypertension	35	M	134	185 100	10	233	Neuroretinitis	365 382	24.8 24.0
Average of sodium determinations in five additional cases									345	
Average of potassium determinations in twenty-three additional cases										20.8

*From the Division of Medicine, Mayo Clinic, Rochester, Minnesota.

exceedingly temperamental, and numerous failures to secure good duplicate readings remain unexplained. The method for the determination of potassium was entirely satisfactory.

TABLE II

SERUM IN HYPERTENSION

CASES	DIAGNOSIS	AGE	SEX	WEIGHT, POUNDS	BLOOD PRESSURE	PHENOLSULPHONEPHTHALEIN EXCRETION IN TWO HOURS	BLOOD UREA, MG. FOR EACH 100 C.C.	FUNDUS OF THE EYE	SODIUM MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A375167	Malignant hypertension	36	M	139	200 / 135	45	47	Cotton wool exudates. Flame hemorrhage	376 379	22.4 21.2
A383725	Arteriosclerosis; hypertension	64	M	215	224 / 104	55	28	Small hemorrhage of right eye	262 240	23.6 23.2
A372113	Arteriosclerosis; hypertension	55	F	141	205 / 90	45	26		375 375	20.3 19.2
A265157	Arteriosclerosis; hypertension; obesity	62	M	235	230 / 125	45		Mild arteriovenous compression	376 375	20.4 22.3
Average of sodium determinations in seven additional cases									361	
Average of potassium determinations in seven additional cases										21.3

TABLE III

SERUM IN DIABETES MELLITUS

CASES	DIAGNOSIS	AGE	SEX	WEIGHT, POUNDS	BLOOD PRESSURE	FUNDUS OF THE EYE	SODIUM MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A377199	Diabetes mellitus	32	M	132	105 / 68	Negative	342 359	22.3 21.6
A372984	Glycosuria	44	M	146	120 / 80	Negative	379 380	22.4 21.9
Average of sodium determinations in three additional cases							514	
Average of potassium determinations in five additional cases								24.5

Because of difficulty with the sodium method, we often failed to secure satisfactory determinations of both elements on the same blood sample. It seemed best, therefore, to tabulate only those cases in which parallel determinations were made, and to give only the total number of determinations and average readings for those cases in which the concentration of only one of the elements was determined.

SUMMARY

Kramer and Tisdall estimated the sodium content of blood serum in normal adults as about 335 mg. for each 100 c.c. of serum, and the potassium content in normal adults as about 19.6 mg. for each 100 c.c. of serum. Our findings for blood serum and spinal fluid are:

Average sodium in blood serum of fifty-six patients with various diseases .....365.0 mg. for each 100 c.c.
Average sodium in cerebrospinal fluid of nine patients with syphilis.....382.0 mg. for each 100 c.c.
Average potassium in blood serum of 118 patients with various diseases ..... 20.7 mg. for each 100 c.c.
Average potassium in cerebrospinal fluid of 117 patients with proved or suspected syphilis.. 10.5 mg. for each 100 c.c.

TABLE IV

SERUM IN SYPHILIS

CASES	DIAGNOSIS	SODIUM, MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A356112	Syphilis of central nervous system, parenchymatous; duodenal ulcer	494 473	22.4 21.2
A370218	Syphilis of central nervous system, late; early changes	341 339	17.3 18.7
A343467	Syphilis of central nervous system, meningeal	355 377	17.2 17.0
A279534	Syphilis of central nervous system	380 379	17.2 17.7
A378803	Taboparesis	528 505	18.5 20.2
A344143	Syphilis of central nervous system; early tabes	365 351	15.7 16.2
Average of sodium determinations in eleven additional cases		385	
Average of potassium determinations in fifty-three additional cases			20.7

We have not found characteristic changes in the sodium or potassium content of the blood serum in the diseases studied. The individual variation in the sodium and potassium content of the blood serum in the same patient from day to day has been as great as that between different patients with different diseases. The potassium values are more constant than those of sodium for blood serum.

The concentration of sodium in the cerebrospinal fluid appears to be approximately the same as that of the blood serum. The potassium content of the cerebrospinal fluid has been found approximately one-half that of blood serum.

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TABLE V

SERUM IN MISCELLANEOUS CASES

CASES	DIAGNOSIS	AGE	SEX	WEIGHT, POUNDS	BLOOD PRESSURE	BLOOD UREA, MG. FOR EACH 100 C.C.	FUNDUS OF THE EYE	SODIUM, MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A378285	Toxic duodenal ulcer under treatment	47	M	134	110 85			192 192	26.3 27.0
A381125	Jaundice; syphilis (?)	27	M	160				294 316	12.6 12.9
A373352	Latent tetany, uterine fibroids; chronic cholecystitis; cholelithiasis	46	F	130	110 70	23		365 366	23.7 24.7
A376444	Cerebral lesion not localized	38	M	194	165 110		Optic neuritis of right	602 596	
A374202		45	M	170	168 80	32	Negative	370 366	
A376493	Angina pectoris	67	M	165	170 80	34	Optic neuritis nous compression	714 718	
A378220	Multiple sclerosis	28	F	114	97 85		Negative	390 407	
A78644	Obesity	58	M	272	145 90			189 206	
A371556	Migraine; latent tetany	38	F	100	130 94		Negative	366 366	
A376079	Chronic myocarditis with decompensation. Auricular fibrillation.	66	M		145 90	44	Mild retinitis; arteriosclerosis	428 416	
A144085	Tetany	37	F	159	110 74	23	Negative	275 253	
A372014	Tetany; gastric (?)	51	F	102	106	41		345 352	
A369991	Upper intestinal obstruction following gastroenterostomy	45	M	138	94 56	264		369 361	
A232879	Jaundice; hepatitis	45	M	155	110 80	28			16.9 18.6 16.8 16.5
A381088	Jaundice; diabetes insipidus	25	M	155	116 80	27	Pale		32.9 32.5

TABLE V—CONT'D
SERUM IN MISCELLANEOUS CASES

CASES	DIAGNOSIS	AGE	SEX	WEIGHT, POUNDS	BLOOD PRESSURE	BLOOD UREA, MG. FOR EACH 100 C.C.	FUNDUS OF THE EYE	SODIUM, MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A144085	Tetany	37	F	159	110	23	Negative		15.8
					74				15.9
A382714	Tetany; physiologic obstruction to duodenum, toxic nephritis, following gastroenterostomy.	41	M	168	110	192			14.9
					70				15.2
A297046	Chronic appendicitis; duodenal ulcer; nephrosis	52	M	157	124	214	Negative		22.4
					98				22.3
A374202	Tonsillar sepsis; duodenal ulcer; dental sepsis; chronic infectious arthritis	45	M	170	108	32	Negative		24.3
					80				22.7
A373608	Bilateral renal tuberculosis; chronic pulmonary tuberculosis (dead)	40	M	148	98	271	Negative		25.7
					58				25.3
A279554	Migraine; constipation	49	F	91	130		Negative		20.2
					80				19.7
A382691	Syphilis investigated								15.6
									15.2

TABLE VI
CEREBROSPINAL FLUID IN SYPHILIS

CASES	DIAGNOSIS	SODIUM, MG. FOR EACH 100 C.C.	POTASSIUM, MG. FOR EACH 100 C.C.
A380056	Syphilis of central nervous system	481	9.2
		489	9.2
A344143	Syphilis of central nervous system	372	9.4
			8.8
A377472	Syphilis of central nervous system	313	10.9
		311	11.1
A343660	Syphilis of central nervous system	406	9.6
		415	10.4
A352833	Syphilis of central nervous system	494	8.7
		467	7.9
A386111	Syphilis	463	11.0
		429	11.2
A356112	Syphilis of central nervous system. Parenchymatous	139	10.2
		179	9.9
A356112		372	11.2
		421	
A379863	Syphilis of central nervous system	354	7.2
		394	7.5
Average of potassium determinations in one hundred eight additional cases of syphilis			10.8

THE PHARMACOLOGY OF BENZYL ALCOHOL AND ITS ESTERS*

II. Some of the Effects of Benzyl Alcohol, Benzyl Benzoate and Benzyl Acetate When Injected Intravenously upon the Respiratory and Circulatory Systems

By Charles M. Gruber, M.D., Ph.D., St. Louis, Mo.

FROM the discordant results obtained by the different investigators and the interpretations of these results it is evident that the pharmacology of the benzyl esters is imperfectly understood.

In recent years Macht has published a series of papers dealing with this subject. In 1918[1] he found that benzyl benzoate and benzyl acetate produced decreased blood pressure when injected subcutaneously, intramuscularly, intraperitoneally or intravenously. This phenomenon he attributed to peripheral vasodilatation. Intravenous injections of benzyl esters tended to depress respiration, and after toxic doses death usually occurred from paralysis of the respiratory center. When benzyl benzoate was added to the perfusion fluid of a perfused excised heart of a cat, increased heart tonus was noted.

During the same year Macht[2] showed that benzyl alcohol lowered blood pressure when injected intravenously in dogs and rabbits. He believes that benzyl alcohol like benzyl benzoate and benzyl acetate produces peripheral vasodilatation, through a direct action on the muscle fibers of the vessel walls without affecting the heart's action or the vasomotor center. He noted that in some instances when given in small doses, benzyl alcohol stimulated respiration but larger doses paralyzed it.

Two years later Macht[3] reported the use of benzyl benzoate in clinical cases where he obtained a decrease in blood pressure. He reiterated his belief that the vasodilator effect was due to peripheral vasodilatation with no involvement of the heart or vasomotor center and that death was due to its paralyzing action upon the medullary centers. He found that an injection of a small dose of epinephrin was not completely effective in bringing the pressure back to normal level.

Mason and Pieck,[4] using a 20 per cent alcoholic solution diluted one-half by adding water, found that benzyl benzoate produces a prompt, pronounced and prolonged fall in blood pressure. This they believe to be due to the action of the drug upon the heart, as they say, "These changes in blood pressure are obviously due to a weakening action of the drug on the heart." They also noted a "passive" decrease in the volume of the kidney and spleen secondary to the profound fall in blood pressure and asphyxia. Paralysis of respiration was observed.

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Without attempting to investigate further the point of action of the benzyl compounds Nielsen and Higgins[5] reported falls in blood pressure and respiratory paralysis after injecting a number of benzyl compounds either dissolved in sweet almond oil or in the form of a water emulsion. They believe the action of these compounds is dependent upon their rate of hydrolysis into benzyl alcohol within the body.

Gruber[6] found benzyl alcohol, benzyl benzoate and benzyl acetate to have no effect upon blood pressure when given orally to dogs.

In view of these conflicting results and theories, numerous aspects of the problem seem to require further study, among them the following:

1. What effects have benzyl alcohol and its esters upon respiration and the repiratory organs?

2. What effects have benzyl alcohol and its esters upon the blood pressure?

3. Do the benzyl esters act equally strongly upon the vessels in all the organs of the body in lowering blood pressure?

4. Do they affect the heart?

METHOD

Thirty-two dogs, 22 cats and 6 rabbits were used in this work, in which over 212 injections were made. The dogs were anesthetized either with ether or with paraldehyde (1.8 ml. per kilo. body weight by stomach). Some of the animals were decerebrated under ether anesthesia and the ether then withdrawn. The cats and rabbits were anesthetized with urethane (2 grams per kilo. body weight by stomach). After anesthetization the animals were tracheotomized.

The respirations were recorded upon the kymograph surface by placing a pneumograph about the thorax connected with a Marey tambour. In all the experiments the blood pressure was taken from a cannula placed in the left carotid artery. In the first 8 experiments upon dogs, a membrane manometer was used to record blood pressure but later it was replaced by a mercury manometer. Sodium carbonate solution was used as an anticoagulant. A chronograph marking 2-, 5- or 30-second intervals was placed at the zero blood pressure level.

The blood pressure in the superior vena cava also was recorded by passing a cannula down the right external jugular vein until it reached the larger vessel. This cannula was connected to a delicate membrane manometer and the system filled with hirud'nized Ringer's solution as an anticoagulant.

The organs of the abdomen were exposed through a mid-line incision. For plethysmographing a loop of intestine, a kidney or the spleen, metal oncometers were used connected with piston recorders which marked changes in volume upon the drum surface. These were applied with as little manipulation as possible and the abdomen then sewed up. A metal leg oncometer connected to a piston recorder was used in recording changes in the volume of the hind limb. The intestine and one other organ were always plethysmo-

graphed simultaneously and in many cases the volume changes of three organs were recorded at once, in addition to the recording of respiration and blood pressure.

After establishing artificial respiration the changes in heart volume were recorded by placing a glass cardiometer about the heart after exposing it by excising the ventral half of the thorax with the loss of as little blood as possible. Changes in blood pressure were recorded simultaneously. In a number of instances records of both auricular and ventricular contractions were made simultaneously with cardiographs applied directly to the heart.

The writing points were placed in a vertical line one above the other.

Several preparations of benzyl benzoate and benzyl acetate were used. These were purchased from the following firms: A 20 per cent alcoholic solution of benzyl benzoate from Hynson, Westcott and Dunning; pure benzyl benzoate from the Eastman Kodak Company and from the Abbott Labora-

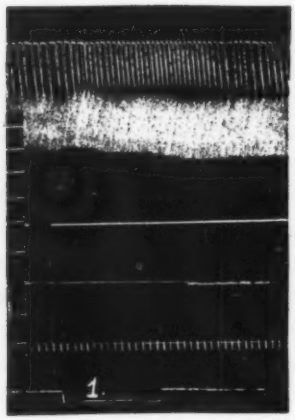

Fig. 1.

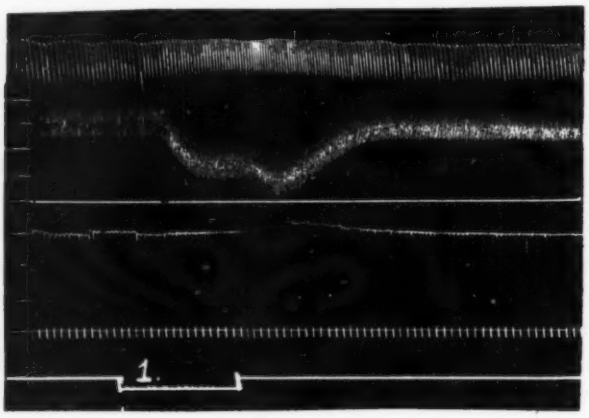

Fig. 2.

Fig. 1.—Dog, 8.5 kilograms. Paraldehyde anesthesia. Top curve respiration, below it blood pressure with membrane manometer. The straight line below it is zero venous pressure with the venous pressure record below. The time is in two second intervals, the writing point also marking atmospheric pressure for the arterial membrane manometer. Bottom line indicates the time of the injection at *1*. All records read from the left to right. Each division of calibration scale of membrane manometer to left of record reads 20 mm. of mercury. *1*,one ml. of benzyl benzoate 20 per cent in alcohol.

Fig. 2.—Conditions the same as Fig. 1. *1*, one ml, benzyl acetate 20 per cent in alcohol.

tories; pure benzyl acetate from Kahlbaum and the Abbott Laboratories. The benzyl alcohol was purchased from Merck & Company.

Benzyl alcohol, benzyl acetate and benzyl benzoate were used in 20 per cent solutions in alcohol, 20 per cent solutions in lecithin Ringer's solution and in 10 per cent acacia Ringer's solution emulsion. The alcoholic solution was used full strength and in solutions diluted with Ringer's solution to make 10 and 5 per cent emulsions. Only a few experiments were performed in which lecithin had been added to Ringer's solution in making an emulsion. The most satisfactory emulsion and that which was used exclusively in the later experiments was a 10 per cent by volume of the drug in Ringer's solution containing 5 per cent gum acacia. In dogs the injections were made

either directly into the left external jugular, the femoral, or the superior mesenteric veins. In cats the injection was made through a cannula placed in the left external jugular vein. The drugs were injected directly into the lateral ear vein in rabbits.

Results

I. RESPIRATION

In nearly all animals benzyl alcohol, benzyl acetate and benzyl benzoate when given intravenously in small and moderately large doses, accelerate the rate and decrease the depth of respiration (Fig. 7). Larger doses produce paralysis of the respiratory center (Fig. 12). The acceleration of respiration was less marked in the animals under deep ether anesthesia but very apparent in the decerebrated dogs and in the urethanized cats and rabbits.

This acceleration in the respiratory rate is well shown in Figs. 1 to 4 inclusive and in Fig. 7. Figs. 1, 2 and 3 were taken from the same animal.

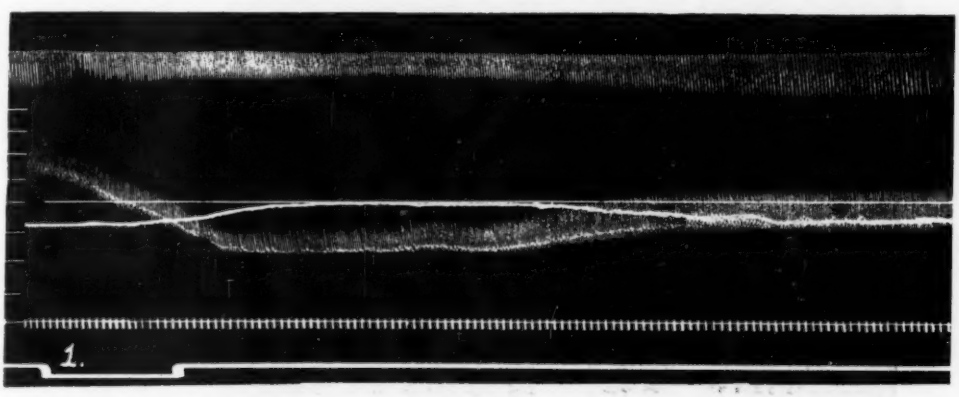

Fig. 3.—Conditions same as Fig. 1. *1*, two ml. benzyl benzoate 20 per cent in alcohol.

In Fig. 1, 1 ml. benzyl benzoate 20 per cent in alcohol was injected in 30 seconds. This injection increased the respiratory rate of 28 to 48 per minute. In Fig. 2, 1 ml. of a 20 per cent benzyl acetate solution in alcohol was injected intravenously in 34 seconds, which increased the respirations from 44 to 88 per minute; and in Fig. 3, 2 ml. of a 20 per cent benzyl benzoate solution in alcohol, injected in 38 seconds increased the respirations from 68 to 96 per minute. In this animal repeated injections of ethyl alcohol in the same amounts as injected in Figs. 1, 2 and 3 caused a slowing of respiration.

Fig. 4 is a record taken from a decerebrated dog. In curve 1, 2 ml. ethyl alcohol 95 per cent were injected and in curve 2, 2 ml. of 20 per cent benzyl benzoate in alcoholic solution were injected. In 1 the respiratory rate was slowed by ethyl alcohol from 34 to 10 per minute. In 2 the respiratory rate was increased from 40 to 62 per minute. The irregularity in depth and rate of respiration in this decerebrated animal was lost for more than 8½ minutes. A subsequent injection of ethyl alcohol 95 per cent slowed respiration.

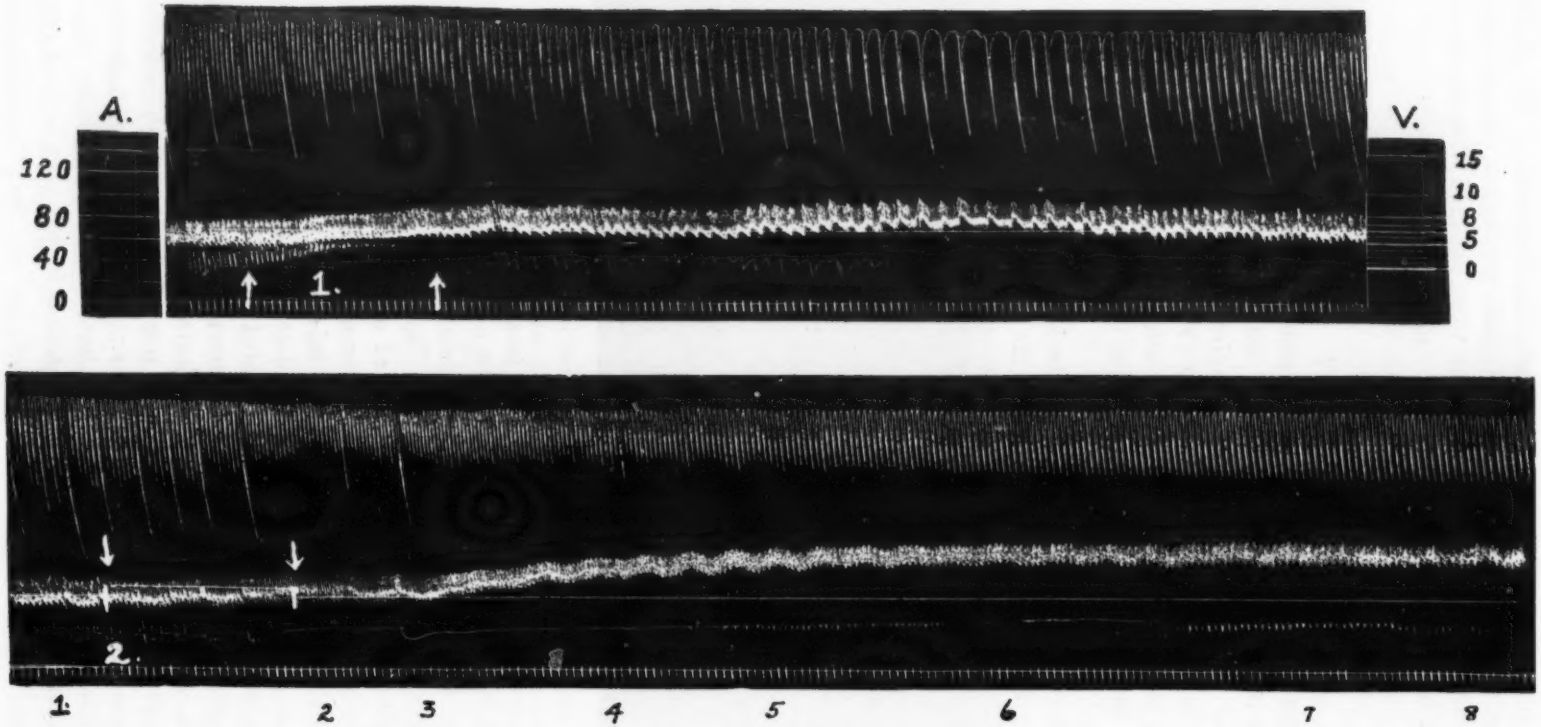

Fig. 4.—Decerebrate dog, 10 kilograms. Top record in each is that of respiration, below it blood pressure with membrane manometer. Middle line zero venous pressure, below it venous pressure taken from superior vena cava. Lowest record time in intervals of 2 seconds and zero blood pressure. Injection made into femoral vein.

A, Calibration of arterial membrane manometer in 20 mm. of mercury for each division; *V*, the calibration of the venous membrane manometer in millimeters of mercury; *1*, two ml. 95 per cent ethyl alcohol; *2*, two ml. 20 per cent benzoate in alcohol.

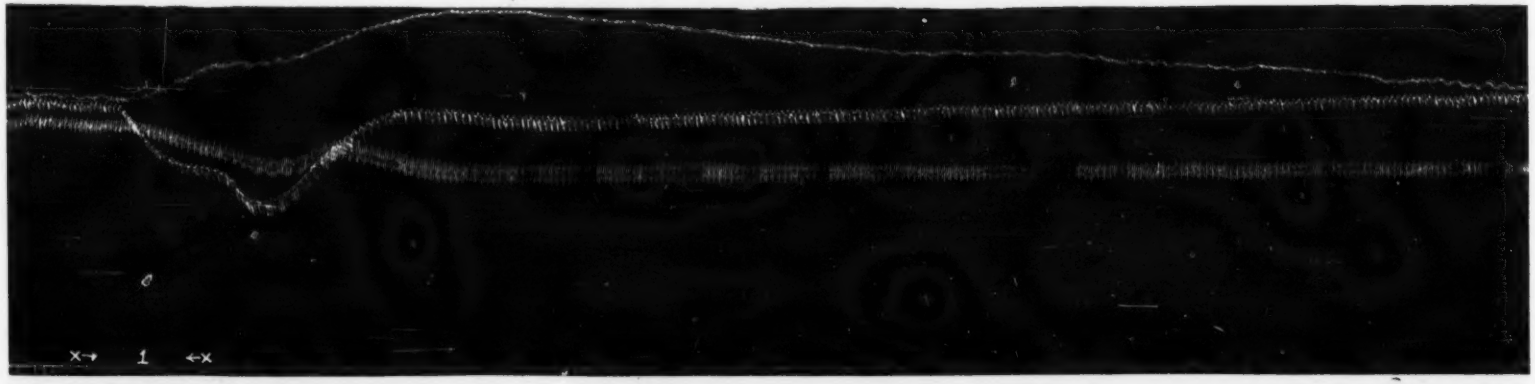

Fig. 5.—Dog, 12. 3 kilograms. Paraldehyde anesthesia. In this and the four following records upward movement of volume records of intestine, kidney, spleen and limb indicates increased volume, downward movement indicates decreased volume. Top record, volume of the loop of intestine. Middle record, blood pressure with mercury manometer, below it volume record of left limb. Lowest record time in 2 second intervals, zero blood pressure and the time and duration of the injection. *1*, two ml. benzyl acetate emulsion 10 per cent in 5 per cent acacia Ringer's solution.

In many animals it was noted that after the respiratory rate was once increased during the experiment it did not return quite to normal. This is seen in Figs. 1, 2 and 3.

In all the animals in which several injections were made the lungs at necropsy showed marked congestion and edema. The bronchioles were filled with a serous fluid. The lower lobes which were most affected would sink when excised and placed in water. This condition existed in dogs, cats and rabbits even though alcohol-free emulsions of the benzyl esters in acacia Ringer's solution were injected into the superior vena cava. The edematous condition did not exist in the three dogs in which the injections were made into the superior mesenteric vein. The size of the animal played an impor-

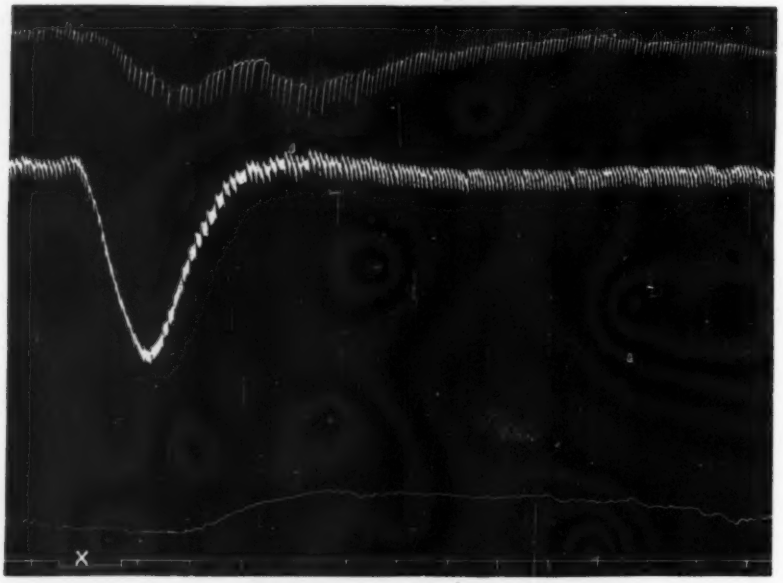

Fig. 6.—Dog, 7 kilograms. Paraldehyde anesthesia. Curare used to abolish all reflexes. Top curve, limb volume, below it the blood pressure with mercury manometer. Lowest curve, time in 30 seconds, point of injection and zero blood pressure—above it the volume of intestinal loop. *x*, 5 ml. benzyl acetate 10 per cent in 5 per cent acacia Ringer's solution.

tant rôle in the rapidity with which embarrassment of respiration developed. In rabbits a few injections would bring about this change, while in large dogs repeated injections for a longer period were necessary. This condition can be explained either upon the basis of increased capillary permeability in the lungs, formation of emboli, or the existence of the two conditions simultaneously.

The author is inclined to attribute this condition to the formation of emboli in the lungs basing his argument on these salient facts: In those animals in which the injections were made into the superior mesenteric vein and hence in which the injected material passed through the portal capillary system before reaching the lungs, the lung conditions did not occur. In the other animals in which the drugs were injected into the external jugular and femoral veins venous pressure gradually mounted and arterial pres-

Fig. 7.—Dog, 18 kilograms. Paraldehyde anesthesia. Top record, respiration; below it volume record of loop of intestine. Middle record blood pressure with mercury manometer; below it, volume record of kidney. Lowest record time interval in five seconds, zero blood pressure and the time and duration of injection. *I*, three ml. benzyl alcohol emulsion 10 per cent in 5 per cent acacia Ringer's solution.

sure showed a gradual fall during the experiments. Mason and Pieck[4] showed that the pulmonary pressure may rise very sharply after an injection of benzyl benzoate. Microscopic examination further favors this view. The lungs showed marked congestion and edema. The edema had extended into the interstitial tissues. There were present fragmentation of nuclei and destruction of many cells. The bronchi were filled with fibrin and a homogenously staining material.

II. EFFECT ON BLOOD PRESSURE

Venous Pressure.—Figs. 1, 2 and 3 taken from the same animal show well the gradual increase in venous pressure during the course of the experi-

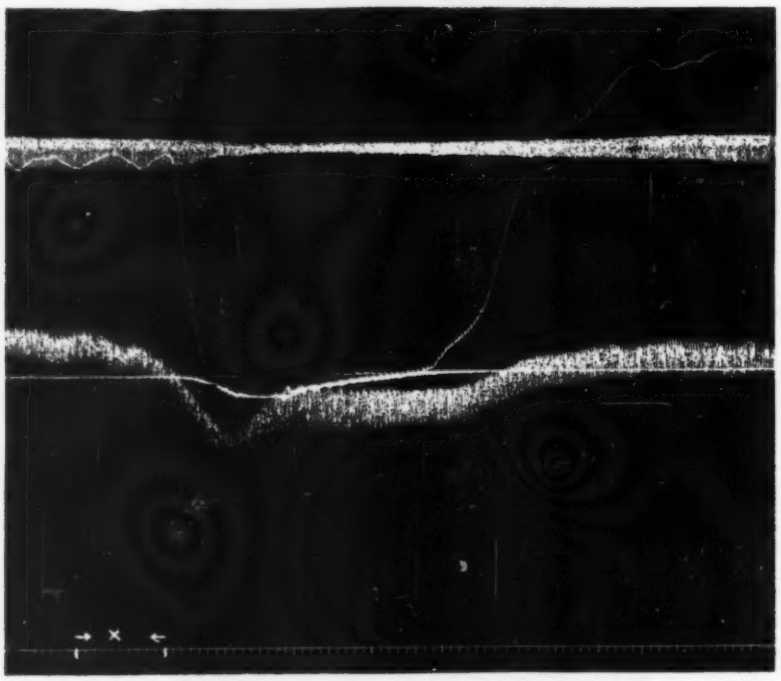

Fig. 8.—Dog, 6.4 kilograms. Paraldehyde anesthesia. Top record, respiration and adjoining it, volume record of spleen. Middle record, blood pressure, mercury manometer and below it the kidney volume. Lowest record, time interval in 5 seconds, zero blood pressure and time and duration of injection. Difference in height of spleen recorded before and after injection is due to the piston recorder's being too small to permit the entire constriction of the spleen to show, so that air was drawn into the system, *x*, 4 ml. benzyl benzoate 10 per cent emulsion in 5 per cent acacia Ringer's solution.

ment with the gradual fall in arterial pressure. In Fig. 1 the arterial pressure was 165 mm. while the venous pressure in the superior vena cava was –8 mm. of mercury. In Fig. 2 taken some minutes later the arterial pressure had dropped to 140 mm. but the venous pressure had increased to –5 mm. of mercury. In Fig. 3 taken still later the arterial pressure had fallen to 115 mm. and the venous pressure is negative by only 4 mm. of mercury. In Fig. 4 the venous pressure increased in spite of the increase in arterial pressure. The gradual increase in venous pressure and lowering of arterial pressure during the experiment suggests, in the face of the postmortem lung findings,

interference of the circulation of blood through the lungs into the left heart. In Fig. 3 the venous pressure became zero after the injection of benzyl benzoate. This was due to the slowing of heart beat and the damming back of the blood into the venous system.

Arterial Pressure.—All observers agree that benzyl alcohol, benzyl acetate and benzyl benzoate when injected intravenously produce a fall in blood pressure; they disagree, however, as to how this change is brought about. In 183 instances in 202 injections these substances produced a lowered blood pressure. In two instances benzyl benzoate and in one benzyl acetate produced no change in blood pressure but an alteration in the volume of the kidney and of the intestine. Fig. 9 is such a record. In two instances benzyl

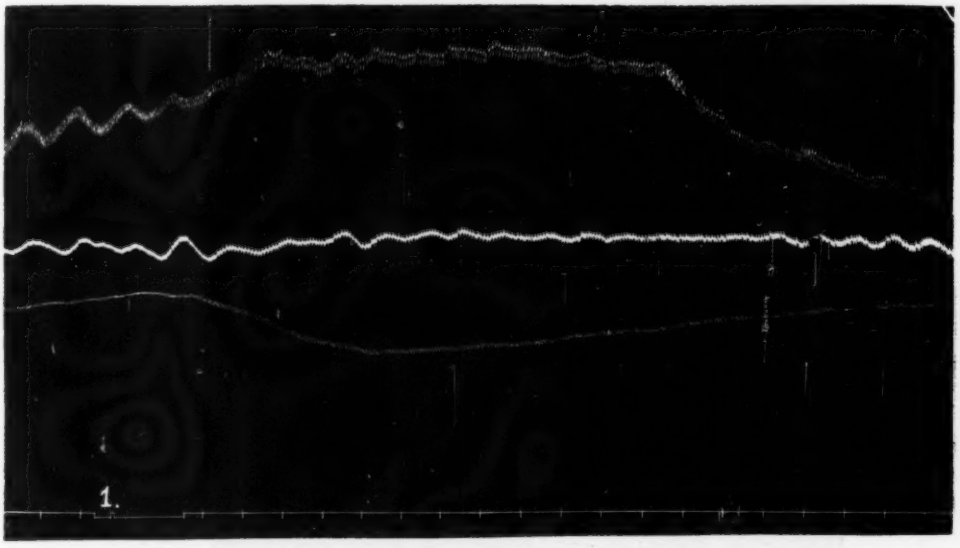

Fig. 9.—Cat, 2.5 kilograms. Urethane anesthesia; adrenals excised. Top record intestinal volume, below it blood pressure with mercury manometer. Lowest record, time in 30 seconds, zero blood pressure and the time and duration of the injection. Above it kidney volume. *1*, five ml. benzyl benzoate emulsion 10 per cent in 5 per cent acacia Ringer's solution.

acetate and in a like number of cases benzyl alcohol produced increases in blood pressure. In the remaining instances, an example of which is Fig. 4, benzyl benzoate produced a pure rise in blood pressure. In Fig. 4, at 1, 2 ml. of 95 per cent ethyl alcohol were injected slowly. The alcohol produced very little change in blood pressure, although it slowed the heart from 195 to 140 beats per minute. In the same Fig. at 2, 2 ml. of a 20 per cent solution of benzyl benzoate in alcohol were slowly injected. As a result of the injection the blood pressure gradually mounted from 70 to 110 mm. of mercury, an increase of 40 mm. This increase lasted for 8½ minutes when it returned to almost the original pressure. The pulse rate was first decreased then increased by the injection. It fluctuated as follows: at 1, 130; 2, 95; 3, 150; 4, 170; 5, 170; 6, 185; 7, 185; 8, 185 beats per minute which latter rate it maintained for the rest of the experiment, a total of 8½ minutes. A subsequent injection of ethyl alcohol, 2 ml. of a 95 per cent solution, had no effect upon blood pressure. The pulse rate, however, is not the only pos-

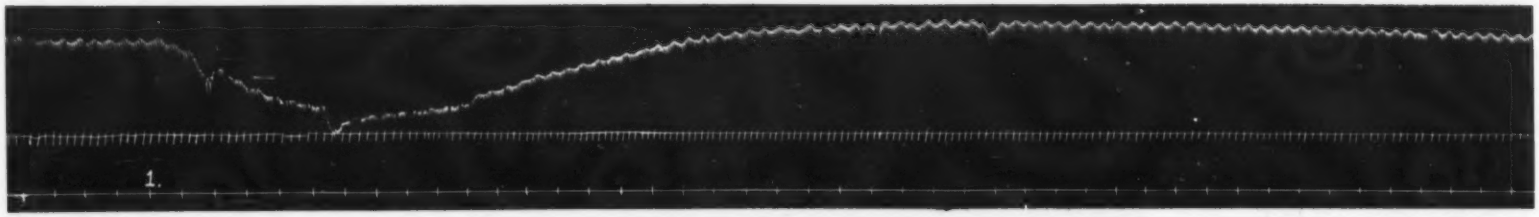

Fig. 10A.—Cat, 2.5 kilograms; urethane anesthesia. Upper record, blood pressure, below it the rate of blood flow in drops from the branch of the mesenteric vein from the loop of intestine. Time in 5 seconds and zero blood pressure. *1*, two ml. benzyl acetate 10 per cent in acacia Ringer's solution.

Fig. 10B.—Cat, 3 kilograms. Animal etherized and exsanguinated. Perfused hind limb. Top record and second below it indicate the rate of flow of perfusion fluid in drops. Below these time interval in 5 seconds. *1* to *x*, 5 ml. blood Ringer's solution saturated with benzyl acetate injected into the perfusion system, *2* to *x*, 5 ml. blood Ringer's solution, ¾ saturation with benzyl alcohol. *3*, 0.5 ml. adrenalin chloride, 1:100,000 solution.

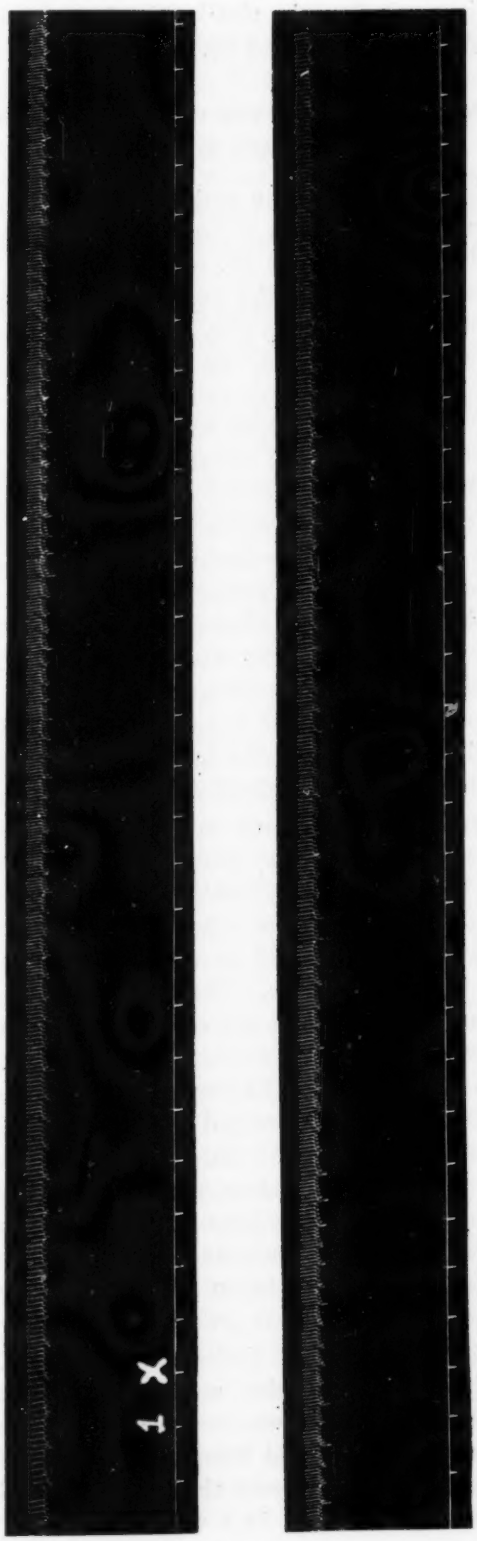

Fig. 10C.—Cat, 3.5 kilograms. Animal etherized and exsanguinated. Perfused kidney. Top record indicates the rate of flow of perfusion fluid in drops, below it the time interval in 5 seconds. *1* to *x*, 5 ml. blood Ringer's solution saturated with benzyl acetate injected into the perfusion system. Lower record continuation of upper.

sible factor involved in producing the rise in blood pressure as will be demonstrated later (see Figs. 5, 6, 7, 8 and 9).

III. EFFECT OF THE BENZYL ESTERS UPON THE VOLUME OF THE INTESTINE, KIDNEY, SPLEEN AND LIMB

That benzyl alcohol, benzyl acetate and benzyl benzoate produce different changes in volume in the different organs can be seen in Figs. 5, 6, 7, 8 and 9. Fig. 5 shows benzyl acetate (2 ml. of a 20 per cent solution in alcohol) to produce marked increase in the volume of the intestine but a decrease in the volume of the limb and in Fig. 6, 5 ml. of benzyl acetate 10 per cent emulsion produced similar effects. In Fig. 7, 3 ml. of benzyl alcohol, 10 per cent in acacia Ringer's solution, caused a small, temporary, passive decrease in volume of the intestine, which was secondary to the fall in blood pressure followed by dilatation of the intestinal loop and decreased volume of the kidney. In Fig. 8, 4 ml. of benzyl benzoate, 10 per cent in acacia Ringer's solution, caused a marked decrease in the volume of the spleen with a slight decrease also in the kidney volume. These changes in volume are not secondary to the change in blood pressure. In most instances the blood pressure had returned almost to the normal height long before the organs resumed their original volume. That the intestine and the kidney can change in volume without a change in blood pressure is observed in Fig. 9. In this animal (cat) 5 ml. benzyl benzoate, 10 per cent in acacia Ringer's solution, produced an increase in the volume of the intestine and a decrease in volume of the kidney without changing the blood pressure.

Because the benzyl esters produced a different volume change in the intestine than in the spleen, the kidney and the limb experiments were performed on 12 cats to determine if their action is different on the vessels of the intestine than on those in the other organs. Five experiments were performed in which a cannula was placed in a branch of the mesenteric vein leading away from a loop of intestine. The drops were recorded by means of tambours. After an injection of 2 ml. of benzyl acetate, 10 per cent in acacia Ringer's solution, the rate of maximum blood flow was increased from 8 to 50 per cent with an average of 25 per cent in the 7 experiments thus performed. In some instances the increased rate of blood flow was preceded by a passive decreased rate due to the fall in blood pressure. Fig. 10-*A* is such a record. At 1, 2 ml. benzyl acetate were injected. There was a passive decrease in the rate of blood flow from 48 to 44 drops per minute which was later increased to 64 drops per minute. This is 33 per cent above the original rate of blood flow. Records taken from the limb vessel showed decreased rate of blood flow in these same animals.

In 7 cats the kidneys and hind limbs were perfused. The perfusion method was essentially the same as that used by Sherrington and Sowton.[7] The free end of the arterial cannula was connected to a rubber tube leading from a reservoir containing a modified Ringer's solution. This solution was made by adding defibrinated blood from the animal to make a 10 per cent blood Ringer's solution. It was kept in a water-bath at 38° C. and oxygen-

ated. The perfusion pressures of 60 mm. of mercury for the limb and 90 mm. of mercury for the kidney were maintained by the rate of flow of oxygen into the perfusion reservoir. The excess oxygen was allowed to escape through a mercury valve which supported a pressure of 60 to 90 mm. of mercury as the experiment demanded. Saturated blood Ringer's solution with benzyl alcohol or benzyl acetate was injected rapidly into the rubber tubing as near the cannula as possible. Such injections had little effect upon the perfusion pressure as this was quickly equalized by the escape of oxygen through the mercury valve.

The drops of perfusion fluid flowing from a glass cannula placed in the vein leading from the organ were recorded on the kymograph by two tambours.

The experiments performed upon these animals show that the action of benzyl alcohol or benzyl acetate is the same upon the blood vessels in the perfused limb and kidney as upon those in the intact intestine; marked vasodilatation, increased number of drops of the perfusion fluid per minute resulted.

Previous perfusion of the organs with apocodein hydrochloride did not alter the response of the blood vessels to the benzyl esters.

Fig. 10-*B* shows the effect of the benzyl esters upon the limb and Fig. 10-*C* their effect upon the kidney. In Fig. 10-*B* the number of drops increased, after injecting 5 ml. blood Ringer's solution saturated with benzyl acetate at 1, from 66 to 156 drops per minute. In the same Fig., at 2, 5 ml. blood Ringer's solution ¾ saturated with benzyl alcohol increased the number of drops from 60 to 120 per minute. In Fig. 10-*C* the rate of perfusion fluid flow from the kidney was increased, after injecting 5 ml. of blood Ringer's solution saturated with benzyl acetate at 1, from 108 to 180 drops per minute.

From the above experiments it would seem that the decreased volume of the kidney and of the limb and probably of the spleen as well, is due either to a passive constriction of the vessels in these organs (fall in blood pressure and drainage of the blood away from these organs, confirmation of Mason and Pieck's[4] work) or to a local compensatory constriction of the vessels in these organs as a result of the fall in blood pressure (an effort on the part of the vasomotor center to maintain a constant pressure). The latter hypothesis is supported by Figs. 5, 6, 7 and 9. In Fig. 5 the maximum decreased volume of the limb and increased volume of the intestine occurred at the time in which the blood pressure had returned to its previous level. In Fig. 6, the blood pressure had returned to its original level some time before the limb and intestine returned to their original volumes and in Fig. 7 the volume changes of the kidney and intestine appear to be independent of the change in blood pressure. In Fig. 9, although the blood pressure did not change, nevertheless the intestine increased in volume at the same time the kidney decreased in volume. In this hypothesis we must assume that the intestinal vessels are more sensitive to the benzyl esters than are those of the kidney and limb and consequently are less influenced by the excitation of the vasomotor center while under benzyl compound influence. Moreover

this organ, the intestine, is supposed to relax under benzyl ester influence, (Macht[1]) which would also favor greater vasodilatation.

That a general compensatory vasoconstriction can result after hemorrhage has been shown by Pilcher and Sollmann[8] and by Gesell.[9] The latter has shown that the blood pressure may be increased after withdrawal of a small amount of blood from dogs. He believes this to be due to an overcompensation by the vasomotor center. It may be possible, therefore, that the rise in general blood pressure observed in some cases after intravenous injections of benzyl esters (Fig. 4) may be due to the increased heart rate and overcompensation by the vasomotor center.

Do the Benzyl Esters Excite the Suprarenal Glands?—Because of the similarity in action between epinephrin and benzyl alcohol, benzyl acetate and benzyl benzoate, it was thought that the benzyl esters might act upon the suprarenal bodies. The latent period in producing the changes would also support such an inference.

In four cats both suprarenal glands were ligated and excised and benzyl alcohol, benzyl acetate and benzyl benzoate injected intravenously in 10 per cent emulsions in acacia Ringer's solution. Fig. 9 is a record selected to show the effect of 5 ml. of benzyl benzoate upon the intestine and kidney volume. Although the blood pressure after this injection did not change as it did in the other animals and in the later injections in this animal, nevertheless it produced vasoconstriction of the kidney (lower curve) and vasodilatation of the intestinal loop (upper curve). In one animal which had but one kidney, benzyl acetate 2.5 ml. of a 10 per cent emulsion in acacia Ringer's solution produced an enormous vasoconstriction in the kidney lasting for seven minutes, but dilatation of the intestinal vessels. The differing actions in different organs by the benzyl esters must be due therefore to the action of the benzyl compound and not to the liberation of adrenalin from the suprarenal glands.

IV. THE EFFECT OF BENZYL ALCOHOL, BENZYL ACETATE AND BENZYL BENZOATE UPON THE INTACT HEART

The injurious effect of the benzyl esters upon the heart muscle is well illustrated in Figs. 11 to 16 inclusive. In Fig. 11, 2 ml. of benzyl acetate emulsion 10 per cent in acacia Ringer's solution was injected intravenously at 1. The auricles (upper curve) continued to beat but feebly and the force of ventricular contraction (lower curve) was reduced but to a much less degree than that of the auricles. This shows the greater toxicity of benzyl acetate upon the auricular tissue. Similar records were obtained with benzyl alcohol and benzyl benzoate.

The marked toxicity of benzyl alcohol is noted in Figs. 12 and 13. In Fig. 12, at 1, 4 ml. of a 5 per cent emulsion of benzyl alcohol in 21 per cent ethyl alcohol and Ringer's solution were injected intravenously. In this animal the heart was stopped for 12 seconds after which it beat rapidly, then was retarded, then gradually increased in rate until its original speed was obtained. In Fig. 13 the animal's heart ceased to beat long before res-

piration ceased. In this animal 2 ml. benzyl alcohol 20 per cent in alcoholic solution were injected. Although artificial respiration was maintained for some time the heart failed to regain its function. In five other animals after injections of comparatively large doses of benzyl benzoate and benzyl acetate the heart ceased beating before respiration stopped.

The hearts of 8 dogs, 4 cats and 2 rabbits were plethysmographed and a total of 32 injections were made. In these, small amounts of benzyl benzoate and benzyl acetate produced decreased heart volume six times and no change in heart volume was observed in three instances. Dilatation of the heart resulted in the remaining number. The quantity tolerated was dependent upon the size of the animal. Large animals responded by decreased volume with much larger quantities than smaller animals. While 0.1 ml. of

Fig 11.—Dog, 10.5 kilograms; paraldehyde anesthesia. Records from above down are auricular contraction, ventricular contraction and time in 5 seconds, and time of injection. Up stroke, diastole; down stroke, systole. *1*, two ml. benzyl acetate emulsion 10 per cent in 5 per cent acacia Ringer's solution.

benzyl acetate 10 per cent produced decreased volume in the rabbit, 2 ml. of the same solution produced the same effect in a large dog.

Fig. 14 is a record in which 6 ml. of benzyl benzoate 10 per cent in acacia Ringer's solution decreased heart volume in a dog weighing 14.8 kilos. In this record there was a temporary increased volume followed by decreased volume of the heart.

These results confirm Macht's[2] findings in the perfused excised cat's heart with benzyl benzoate in which increased tonus was observed.

Figs. 15 and 16 are plethysmograph records of the heart of a dog and a cat respectively. In Fig. 15, 2.5 ml. of a 10 per cent emulsion of benzyl alcohol in acacia Ringer's solution were injected intravenously at 1. The volume of the heart almost instantly increased with some slowing of its rate

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of beat. In Fig. 16 the animal was treated in the usual manner and at 3, 3 ml. of benzyl acetate emulsion of 10 per cent in acacia Ringer's solution were injected intravenously. The heart slowed and showed paroxysmal flutter approaching fibrillation from which it gradually recovered. There can be little doubt but that the fall in blood pressure from these drugs can be markedly influenced by their action upon the heart.

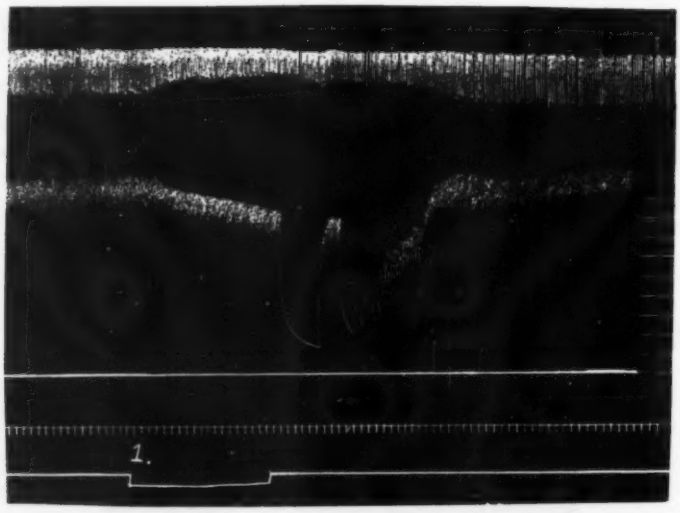

Fig. 12.—Dog, 7 kilograms; ether anesthesia. Reading curves from top down: respiration, blood pressure with membrane manometer, zero line for blood pressure, time in 2 seconds and line showing time and duration of injection. *1*, one ml. benzyl alcohol 20 per cent in alcohol diluted with 3 ml. Ringer's solution.

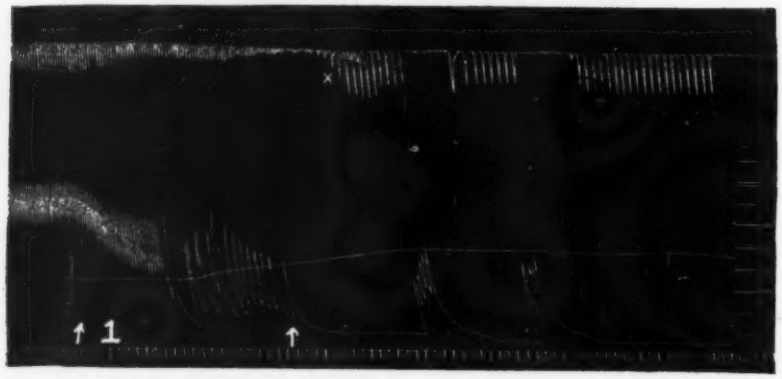

Fig. 13.—Dog, 9 kilograms; ether anesthesia. Reading records from top down: respiration, blood pressure with membrane manometer, venous pressure, zero arterial blood pressure and time in 2 second intervals. Artificial respiration at X. Between the arrows at *1*, 2 ml. benzyl alcohol 20 per cent in alcohol were injected.

Relative Toxicity.—It was found that benzyl acetate is much more toxic to the heart and other organs than is either benzyl alcohol or benzyl benzoate, when injected in a 10 per cent emulsion in acacia Ringer's solution by volume. One ml. of benzyl acetate was found to have as much effect as 2.5 ml. of benzyl alcohol and 5 ml. of benzyl benzoate of the same volume per cent. If, however, these substances are dissolved in ethyl alcohol the

latter two, at least, are more toxic, the three thus becoming of about equal toxicity.

Are the actions of benzyl acetate and of benzyl benzoate dependent upon their hydrolysis in producing their effects?—Nielsen and Higgins[5] claim that the difference in rate of action of benzyl compounds in the body is dependent upon their rate of hydrolysis. Volwiler and Vliet[10] found 5 per cent of benzyl benzoate and 12 per cent of benzyl acetate to be hydrolyzed 30 minutes after these substances were alkalinized and kept at 20° C. After making 45 injections of benzyl alcohol, 91 injections of benzyl acetate and 76 injections

Fig. 14.—Dog, 14.8 kilograms; paraldehyde anesthesia. Top record, heart volume; below it blood pressure with mercury manometer. Time interval in 30 seconds, zero blood pressure and point of injection. When piston recorder lever was horizontal the mercury manometer and time marker wrote 1 cm. in advance of it. In this and the remaining records upward movement of the writing point of heart volume indicates dilatation of heart; downward movement indicates decreased volume. *x*, 6 ml. benzyl benzoate 10 per cent in 5 per cent acacia Ringer's solution.

of benzyl benzoate, we found no evidence whatever indicating that the effects of benzyl acetate and benzyl benzoate in the body were dependent upon their rate of hydrolysis into benzyl alcohol. The time of the appearance of the effect was the same in each but the degree differed according to the substance used as given above. There is no indication that these substances act only after hydrolysis.

Macht[11] found the benzyl esters to be much more effective in rabbits than in dogs in lowering the blood pressure when injected subcutaneously

and intramuscularly. This difference he explains upon the basis of more rapid absorption in rabbits. Another factor should be taken into consideration and that is the size of the animal. It was found, e.g., that 0.5 ml. of a 10 per cent emulsion of benzyl acetate in acacia Ringer's solution when injected intravenously produced as marked an effect in the rabbit as 5 ml. in a medium-sized dog and 2 ml. in the cat.

SUMMARY

1. Forty-five injections of benzyl alcohol, 91 of benzyl acetate and 76 of benzyl benzoate were made intravenously in 32 dogs, 22 cats and 6 rabbits.

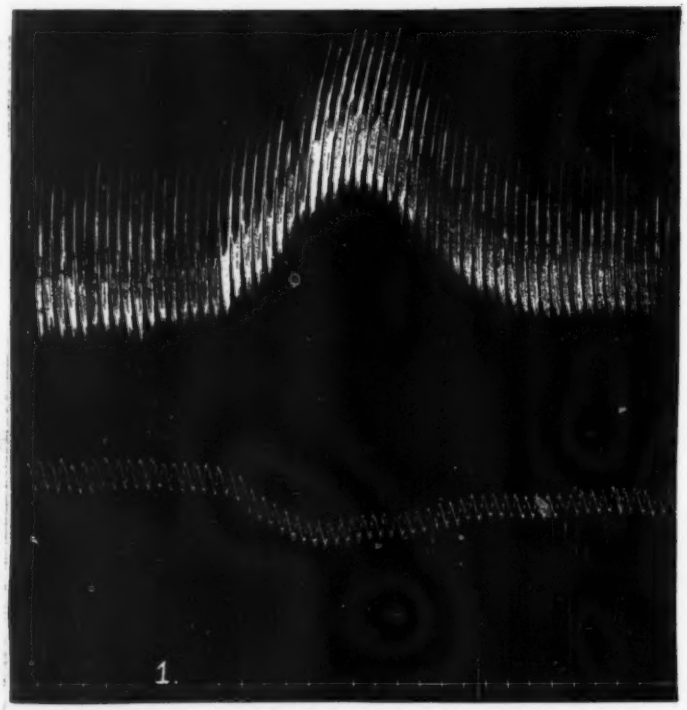

Fig. 15.—Dog, 17 kilograms; paraldehyde anesthesia. Top record, heart volume, middle, blood pressure, bottom, time in 5 seconds, zero blood pressure and time of injection. *1*, 2.5 ml. benzyl alcohol 10 per cent emulsion in 5 per cent acacia Ringer's solution.

2. Moderately large doses of the benzyl esters accelerate respiration, but decrease the depth; large doses paralyze the respiratory center.

3. Repeated injections cause edema of the lungs, fluid in the bronchioles and marked passive congestion of the lower and middle lobes due probably to emboli.

4. Repeated injections of benzyl esters cause a permanent increase in the pressure of the superior vena cava and lowered arterial pressure.

5. A moderate dose of any of the above benzyl esters acutely increases temporarily the pressure in the superior vena cava.

6. The various benzyl compounds in comparatively large doses lowered blood pressure in 183 instances. Moderate doses had no effect three times and produced a rise in blood pressure 16 times.

7. In 16 dogs and 6 cats benzyl alcohol, benzyl acetate and benzyl benzoate caused increased volume of the loop of intestine 46 times in as many experiments. They decreased the volume of the kidney 30 times, of the limb 14 times and of the spleen 5 times.

8. Benzyl compounds are more toxic to the auricles than to the ventricles.

9. Benzyl alcohol, benzyl acetate and benzyl benzoate may paralyze the heart muscle before there is paralysis of the respiratory center.

10. Benzyl compounds can accelerate the heart as well as slow it.

11. Benzyl alcohol, benzyl acetate and benzyl benzoate in 32 injections

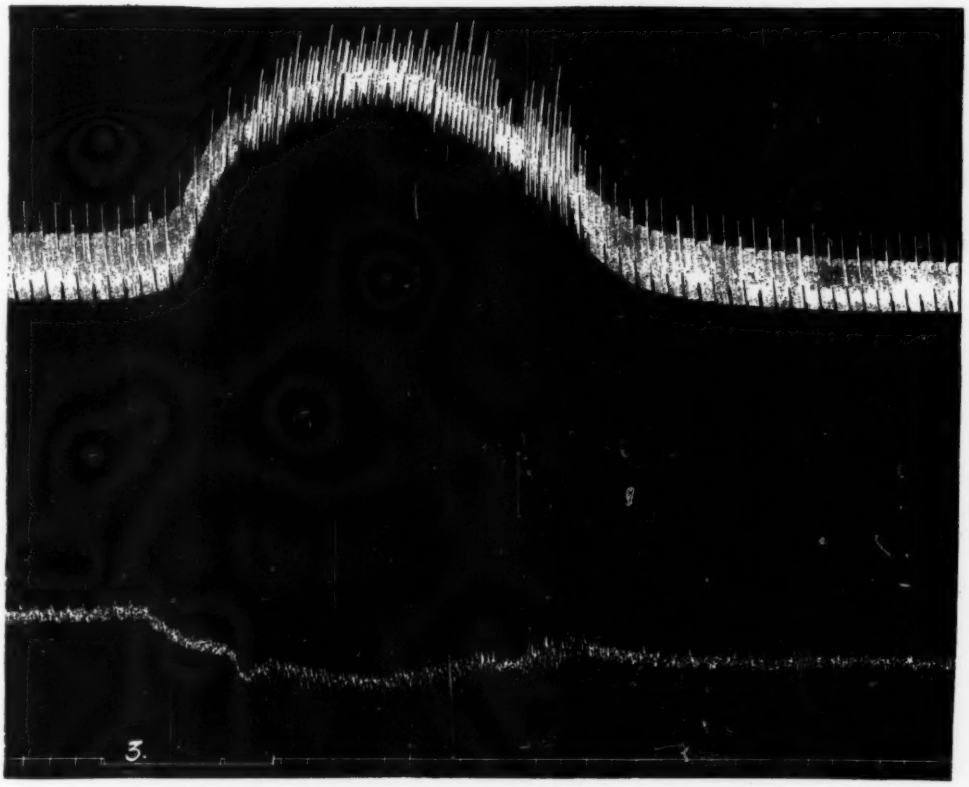

Fig. 16.—Cat, 2.5 kilograms; urethane anesthesia. Top record, heart volume, middle, blood pressure; bottom, time in 5 seconds, zero blood pressure and the time of injection. *3*, three ml. benzyl acetate 10 per cent emulsion in 5 per cent acacia Ringer's solution.

into 8 dogs, 4 cats and 2 rabbits, gave the following results: Decreased volume of the heart in six instances, and increased volume in the remainder. Paroxysmal flutter approaching fibrillation, pulsus alternans and trigeminal pulse were observed after large doses.

12. With the same volume per cent, 1 ml. benzyl acetate 10 per cent emulsion in acacia Ringer's solution was found to be as toxic to the heart and other organs as 2.5 ml. benzyl alcohol and 5 ml. benzyl benzoate.

13. The actions of benzyl acetate and benzyl benzoate are not dependent upon their hydrolysis into benzyl alcohol.

14. One-half ml. of benzyl acetate 10 per cent emulsion in acacia Ringer's solution was found to be as toxic to a rabbit's heart as 5 ml. of the same solution were to the heart of a dog weighing 14 kilos.

15. Benzyl esters have the same action upon the blood vessels in the perfused kidney and limb (vasodilatation) as they have upon the vessels of the intact intestine.

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CLINICAL AND LABORATORY PROCEDURES IN PEDIATRICS

By A. Levinson, M.D., Chicago, Ill.

(Continued from page 44.)

Intubation, Extubation and Tracheotomy

If laryngeal diphtheria is recognized early and sufficient antitoxin is given, there is no need of intubation. When the disease has advanced to the stage of cyanosis, retraction of the lower part of the sternum and gasping for breath, the laryngeal obstruction should be relieved by mechanical means. Intubation is preferable to tracheotomy, as it produces no open wound nor does it leave a scar. When intubation fails tracheotomy should be employed.

Two types of intubation instruments are used in America: the O'Dwyer

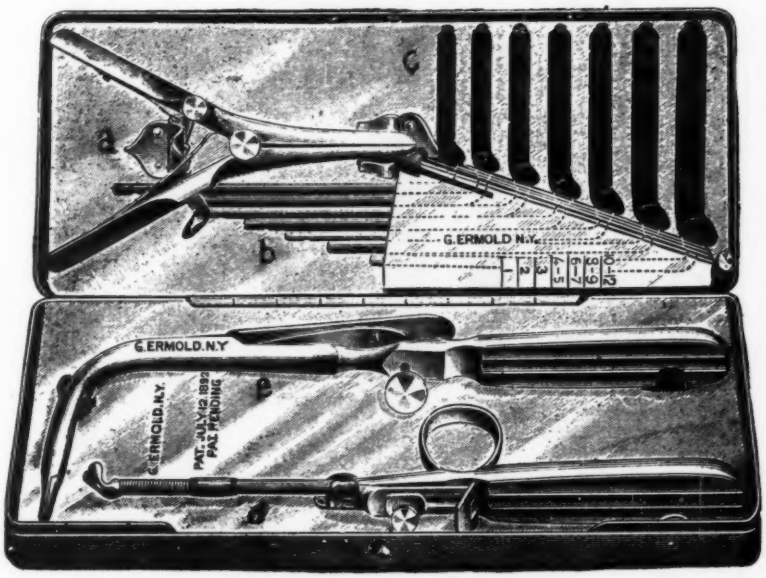

Fig. 52.—Ermold O'Dwyer intubation set: *a*, mouth gag; *b*, obturators: *c*, hard rubber tubes; *d*, introducer; *e*, extubator.

and the Feroud. The first (Fig. 52) is made of hard rubber lined by metal and the latter is made of metal. The Feroud is more simple in that no special obturator is used for each tube, and that one instrument is used both for intubation and extubation (Fig. 53).

INTUBATION

The patient, wrapped in a sheet, to prevent struggling, is placed on a table, the head being brought to the end of the table, and a small pillow placed under the neck to bring the larynx into prominence. The body of the child is held by one assistant and the head is steadied firmly by another

assistant. A mouthgag is placed in the child's mouth and held firmly by the assistant holding the child's head. The operator, standing at the right hand side of the patient, facing him, introduces the index finger of his left hand into the patient's pharynx and pulls the epiglottis toward him, so as to uncover the larynx.

The tube attached to the end of the introducer held in the right hand and controlled by means of a string stretching from the tube to the hand and wrapped around the finger, is now introduced into the larynx guided by the index finger of the left hand. When the tube is already in the larynx, the introducer is removed and the thread is cut. The mucus in the pharynx is now wiped out by means of gauze and the mouthgag is removed. A metallic cough is a good indication that the tube is in the larynx and not in the esophagus. An improvement in the cyanosis and an improvement in breathing is another good indication, that the tube is in the larynx.

Fig. 53.—Feroud's intubation set: *a*, mouth gag; *b*, introducer and extractor in one piece; *c*, gold plated metal tubes; *d*, scale of years for measuring the sizes of the tubes.

Every intubated patient should be watched constantly by a competent nurse, and a physician experienced in intubation should be at hand, so that in case the tube gets clogged up, the child may be extubated without delay, and if the tube is coughed up and the child becomes cyanotic, reintubation may be performed. For this reason it is always best to treat intubated patients at a hospital for contagious diseases, instead of at home.

Feeding of an intubated patient is best accomplished by the Casselberry method, which consists of lowering the patient's head below the level of the rest of the body while administering food. An intubated patient needs a great deal of fresh and warm air. A croup kettle may be of some value, but is not always necessary. Ordinary room temperature will usually answer the purpose. Above all it must not be forgotten that all intubated cases must have diphtheria antitoxin to counteract the disease. Intubation serves

only as a means of keeping the larynx open for a short while, but does not cure the disease.

The tube, if not coughed up, should be left in the larynx for three days and then removed. If the patient cannot get along without the tube he must be reintubated. If the tube is coughed up by the patient before three days, the patient should be watched closely and if he can get along without a tube he should be let alone, otherwise he should be reintubated.

EXTUBATION

Experts often remove the tube by making pressure on the lower part of the larynx. The child then spits the tube out. Some physicians leave the thread in the tube in every intubated case, so as to be able to pull the tube

Fig. 54.—Intubation outfit.

out easily. This, however, is not always advisable, as the child, unless watched constantly, may pull the tube out himself. If neither of the above methods is employed, extubation has to be done by means of an extubator. The preparation is the same as for intubation, and the extubation is performed by pulling the epiglottis toward the operator, introducing the instrument into the tube, and pulling the tube upwards and forwards. An extubated child should be watched carefully, as it may need reintubation very soon.

TRACHEOTOMY

The open laryngeal operation or tracheotomy should be resorted to only after all other means of supplying air to the patient have failed. Such, for

instance, may be the case when the obstruction is not only in the larynx but also in the pharynx in which case intubation will do no good. Such may also be the case when there is so much membrane in the larynx that the intubation tube cannot go through at all.

The instruments required for tracheotomy are: (1) tracheotomy tube; (2) scalpel; (3) blunt hooks; (4) needle and catgut or silkworm gut. In time of emergency one can get along with a tracheotomy tube and scalpel. A pocket knife and the hard rubber or metal tip of a stethoscope have been known to save a life during emergency.

No anesthetic is required. In fact a general anesthetic is usually counter-indicated. A local anesthetic may be used.

TECHNIC.—The patient is placed in the recumbent posture with the head at the edge of the table. A sand bag or a rolled towel is placed under the neck so as to bring the trachea into prominence. The patient's head is steadied by an assistant.

After ascertaining the position of the thyroid body and of the cricoid cartilage, an incision is made through the skin, subcutaneous fascia, and

Fig. 55.—Tracheotomy tubes.

anterior layer of the cervical fascia. The sternohyoid and sternothyroid muscles are then separated by the blunt side of the scalpel. The isthmus of the thyroid is held downward by a blunt hook. The edges of the wound are also kept separated by hooks. The fascia of the trachea is now cut, exposing the tracheal rings. Two rings of the trachea are now cut with a scalpel held in the right hand, while the trachea is held tense with the left hand. A rush of air and often also of mucus into the wound indicates that the trachea has been cut. The cartilage is now separated and the tracheotomy tube introduced inward and downward. The tape bands on each side of the tube are tied around the patient's neck so as to hold the tube in position. A sterile piece of gauze at the base of the tube, next to the wound, helps to keep the dust out. No sutures are necessary as a rule, but if the wound is large, one or two sutures may be necessary.

Tracheotomized patients should receive extraordinary care. The inner tube should be removed and cleaned every few hours, or as soon as it clogs up. The gauze around the wound should be changed every day and the outer tube should be removed every two days. The air in the patient's

room should be fresh and warm. No croup kettle, however, is necessary. The tube should be left in four to six days unless the patient cannot get along without it.

Thoracentesis

Pleural Fluid

Infants and children develop fluid in the pleural cavity quite frequently after pneumonia. Contrary to the adults, in whom the fluid is usually caused by the tubercle bacillus, the pleural fluid in children's chests is usually thick and is caused by pneumococcus or streptococcus and less frequently by the influenza bacillus and still less frequently by the tubercle bacillus. Because of the frequency of empyema in children and because of the serious symptoms produced when a large quantity of fluid accumulates in the chest, thoracentesis should be done on mere suspicion of fluid in the chest. The procedure is harmless and of great value.

The following instruments are necessary for the procedure:

1. Needle 16 to 18 gauge.
2. Syringe.
3. Sterile tubes for collection of material for culture.
4. Slide for smear.

Technic.—The child is held firmly either in the recumbent or in the sitting posture. The dullest spot on the posterior chest is selected. As a rule the spot immediately below the lower angle of the scapula will be found most suitable. The area is washed with iodine and alcohol and a sterile towel is applied above and below the area of operation. Ethyl chloride may be used as a local anesthetic, but no general anesthetic must be used, the latter being dangerous. The needle, attached to the syringe is now introduced for ¼ to ½ inch and the plunger of the syringe withdrawn carefully. If fluid appears the plunger is withdrawn still further. If no fluid appears the needle should be directed upward or downward, as the pus may be localized in one area.

After the pus is obtained the needle is withdrawn and the wound is closed with collodion. The pus is to be examined in direct smear and also cultured for the causative organisms.

Repeated punctures may be performed for therapeutic purposes.

Pericardial Puncture.—Clinical and pathologic investigations have disclosed the fact that infants and children suffer quite often from pericardial effusions. Endeavor should always be made to establish the diagnosis of pericarditis by percussion and auscultation. If this fails a pericardial puncture may be done. The puncture may also serve to withdraw the exudate for therapeutic purposes.

Technic.—The instruments necessary are the same as for pleural puncture. The child is placed in the recumbent posture. The cardiac area is washed with alcohol and iodine. The needle is introduced in the fourth or fifth interspace 2 to 2½ inches to left of the sternum, and the piston of the

syringe slowly withdrawn. If the needle moves in the chest like a pendulum, it is in the heart proper and should be withdrawn. If the needle does not move with the heart beats the plunger should be carefully withdrawn, and the barrel of the syringe watched for pus or serous fluid.

Milk

Human milk seldom requires chemical or bacteriologic examination. It may be taken for granted that a healthy mother has healthy milk. It has been found that with the exception of tuberculosis and syphilis the diseases of the mother do not affect the baby. There are some diseases of the mother besides tuberculosis and syphilis that contraindicate nursing, such, for instance, as carcinoma, diabetes, endocarditis, but this is not because of the disease being transmitted to the child, but because nursing would exhaust the mother. The composition of human milk does vary in different individuals and in various stages of lactation. The difference, however, is, as a rule, not sufficiently great to make examination worth while for clinical purposes. In cases of exudative diathesis, it may occasionally be wise to examine the fat content. In such cases, however, a large sample should be taken before nursing and after nursing.

The quantity of human milk may be determined by weighing the child before and after nursing and also by watching the child's weight curve for a period of days or weeks.

Cow's milk should be examined bacteriologically and chemically. The bacterial count in any milk given to babies should not exceed 50,000 per cubic millimeter. The technic of counting bacteria in milk is described in text books on bacteriology and on milk. When no expert bacteriologic examination can be had the milk may be examined by the clinician by putting a drop of it under the microscope and counting the number of clumps of bacteria and the number of cells, especially phagocytes, in it. In time of epidemics of diphtheria, scarlet fever, streptococcus sore throat, typhoid fever, or dysentery the milk should be cultured for the respective bacteria. Instruction for collection of samples may be obtained from various boards of health. Milk may also have to be examined for tubercle bacilli and goat's milk for malta-fever organisms.

The fat should range between 3 and 4 per cent, unless the infant's condition requires a very low fat. The Babcock method is used extensively for this purpose. The sugar should vary between 3 and 4 per cent.

Milk given to babies should be obtained from healthy cows. The tuberculin test should be performed on all cows supplying milk for babies. The milkers should also be free from disease and should exercise the utmost cleanliness. For this reason certified milk, namely milk obtained from cows supervised by a special medical committee, which sees that the milk is obtained from healthy cows by healthy attendants and delivered in sealed bottles the day of milking, is preferable. Next to certified milk boiled milk is most useful, as boiling destroys the bacteria. Some pediatricians oppose the boiling of milk, because it destroys the vitamines. The latter, however,

can be supplied by the addition of fruit juices, notably orange juice, tomato juice, and lemon juice. Pasteurized milk is used extensively all over the United States.

Basal Metabolism

The determination of basal metabolism is important in the diagnosis of hypothyroidism in which case the basal metabolism is decreased, and hyperthyroidism in which case the basal metabolism is increased. It is usually difficult to keep a child at rest during the examination. One must therefore allow for a certain percentage of error. However, if the final reading registers 15 per cent below or above, the evidence should be considered strongly corroborative.

Electrical Reaction

Hyperexcitability of an infant toward a galvanic current is considered the most important sign of spasmophilia or tetany (Erb's sign).

In normal infants 6 to 9 milliamperes are required to obtain a cathodal opening contraction (COC), 5 to 6 milliamperes for an anodal opening contraction (AOC). In children over two years AOC may be obtained with less than 5 milliamperes; 3 to 4 milliamperes for an anodal closing contraction (ACC), and 2 to 3 milliamperes for a cathodal closing contraction (CCC).

In spasmophilic infants, both AOC and COC may be obtained with less than 5 milliamperes, at times even with 1 milliampere. It can easily be seen that COC is the most important electrical reaction for the diagnosis of spasmophilia.

Method.—A galvanic current from a small dry cell battery may be used. A flat electrode is placed on the infant's chest or abdomen and a small round electrode is placed at the bend of the elbow (median nerve) or in the outer portion of the popliteal space (peroneal nerve).

Contractions of the fingers (in case of median nerve irritation) and of the toes (in case of peroneal nerve irritation) are looked for with cathodal opening. The pole is then reversed and anodal opening contractions are looked for. At first a very weak current is used, starting with 1 to 2 milliamperes. When no cathodal opening contraction is obtained, the current is increased till a contraction is obtained.

VARIATIONS OF THE PERCENTAGE OF HEMOGLOBIN IN MAN DURING THE DAY*

By I. M. Rabinovitch, M.D., Montreal, Quebec

THE interpretation of the variations in the amount of hemoglobin in the circulating blood is liable to errors of various kinds. As in the determination of the value of any other physical property, the chief sources of error are (a) constant errors, due to the apparatus or methods employed, and (b) accidental errors, due to the personal equation of observations. Constant errors due to the apparatus, or varying the method in the estimation of hemoglobin percentage have been practically eliminated. It is apparent from the many methods described that much difficulty has been met with. Those in common practice include the application of the acidhematin principle (Sahli, Newcomer), the Hoppe-Seyler carbon monoxide principle (Haldane, Palmer), the Robschiet method (Palmer procedure applied to Sahli principle), and the direct color comparisons (Dare, Gower, Talquist, Dreyer). Recently a method has been described based on the determination of the iron content of the blood (San Yin Wong). The difficulties met with in all these methods have been, chiefly, the rate of formation of color, the apparatus, and the stability of standards. The greatest difficulty has, however, been in the errors of observation—the personal equation, unavoidable in all colorimetric procedures. To overcome these errors repeated observations have been suggested, accepting the arithmetical mean of all results. There is very little doubt, however, that both sources of error—constant and accidental—have been reduced to the minimum by the introduction of the simple gasometric method, based upon the oxygen carrying capacity of the blood (Van Slyke). With the introduction of this method a little more blood is required for the determinations. This has a distinct advantage. The direct method (venous puncture) of obtaining this increased quantity of blood has eliminated a not uncommon source of error met with in obtaining cutaneous (capillary) blood. It is common knowledge that a free flow of blood undiluted with lymph is not always obtained, unless the needle is rapidly inserted, squeezing out is not practiced, and cold or congestion is avoided. The method of Lundsgaard and Möller (1922) of obtaining cutaneous blood yields results which approximate more closely those obtained from samples obtained by venous puncture. This is shown in Table I, where the observations made on three individuals are recorded. The method used in each case was the same (Palmer). In column (a) are shown the hemoglobin values of the blood obtained by the usual cutaneous puncture; column (b) those

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of the blood obtained by the Lundsgaard procedure; and column (c) those of the blood obtained directly by venous puncture and oxalated.

Since all three samples, a, b, and c, were obtained from the same individual within a very short space of time (5 minutes), the factor of dilution with lymph by the usual procedure is strongly suggested.

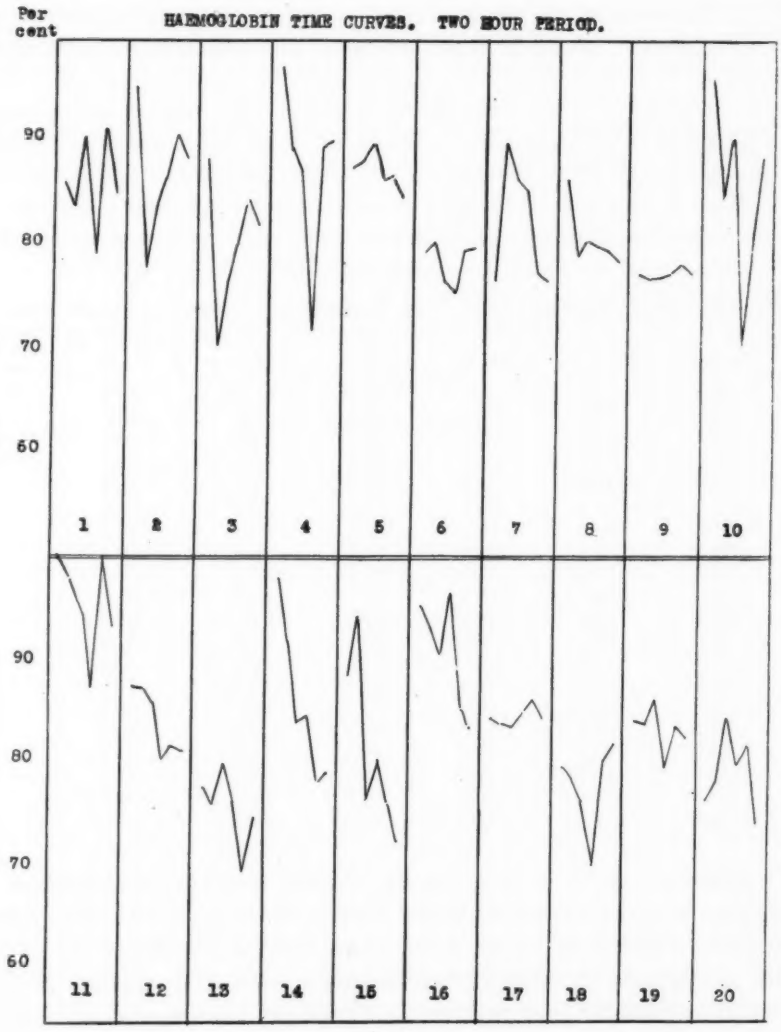

Chart I

With the elimination of all the sources of error referred to above, there is yet one not generally recognized in the interpretation of values; the possible diurnal variation. The literature on this phase of the subject is very scanty. The only observations within recent years are those of Dreyer.[1] A part of these observations are corroborated here. The object of this preliminary report is to present only the data obtained showing the variations in the percentage of hemoglobin. More complete data including cell counts,

color index, blood volume and possible factors governing such variations, form part of observations made to be reported upon later.

The clinical importance of an accurate knowledge of the hemoglobin content of the blood in the various forms of anemia is well recognized. Judging from the enormous amount of work done during the last few years, since the introduction of practical microchemical and physical laboratory methods, the importance of studies of the blood as a chemicophysical system is well recognized. Many conclusions and generalizations have been re-

TABLE I

SUBJECT	A	B	C
1	80	86	87
2	81	86	89
3	84	89	92

TABLE II

HEMOGLOBIN PERCENTAGE VARIATION OBSERVED AT TWO HOUR INTERVALS

NO.	8 A.M.	10 A.M.	12 N.	2 P.M.	4 P.M.	6 P.M.	MAXIMUM VARIATION
1	86.2	84.0	90.8	79.2	91.2	85.0	12.0
2	95.4	78.0	84.0	86.8	91.0	88.3	17.4
3	88.4	70.2	76.7	80.3	84.6	82.0	18.2
4	97.4	89.3	87.1	71.4	89.9	90.2	26.0
5	87.6	88.2	90.0	86.5	87.0	84.8	5.2
6	79.3	80.4	76.7	75.9	80.0	80.0	4.5
7	76.9	90.0	86.7	85.3	77.2	76.8	13.2
8	86.4	79.0	80.4	80.0	79.8	78.3	8.1
9	77.2	76.9	77.0	77.3	78.1	77.2	1.1
10	96.3	84.6	90.2	70.1	80.3	88.7	26.2
11	100.2	98.0	94.2	87.1	100.0	93.2	13.1
12	87.6	87.5	86.0	80.4	81.8	81.1	7.2
13	77.8	76.0	80.0	76.3	69.6	74.7	10.4
14	98.2	92.0	84.0	84.3	78.0	79.0	20.2
15	88.6	94.3	76.1	80.4	76.3	72.9	21.4
16	95.1	93.2	90.6	96.2	85.1	83.7	12.5
17	84.6	84.0	83.9	85.0	86.2	84.2	2.3
18	79.8	78.6	76.4	70.2	80.1	82.0	11.8
19	84.2	84.0	86.3	79.9	83.7	82.7	6.4
20	76.9	78.2	84.3	80.0	82.0	74.0	10.3

corded. Some of these will probably require further qualification, when consideration is given to the possible degree of variation in the percentage of hemoglobin which may occur in the same individual during the same day. That the percentage of distribution between cells and plasma is different for different substances is recognized. Threshold studies are made either on plasma or whole blood, depending upon the substance under consideration. The extent of ionic interchange between corpuscles and plasma is an important factor in respiratory exchange and acid-base equilibrium studies. Here, separation of plasma may not only be necessary, but special precautions, time factor, etc., must also be taken to preclude any change in the distribution of ions during the separation. Even in some instances where a substance under consideration is regarded as being equally distributed between the cells and plasma, this equal distribution is held in doubt by some and regarded to be due to the method employed to prevent clotting. Thus,

to oxalate has been attributed the injuring of the cell membrane altering its permeability. The resistance the corpuscular membrane offers to the passage of charged ions, and therefore electrical currents, forms the basis of a method used in determining the relative volume of plasma and cells. The greater the number of cells contained in a given specimen of blood the lower will be the conductivity. These few illustrations suggest the possible influence variations in the amount of hemoglobin in the blood, if they do occur to a marked degree, may have in the interpretation of various problems.

Table II shows the data obtained in twenty normal individuals. These are represented graphically in Chart 1. All determinations were made with the Van Slyke apparatus, and the per cent of hemoglobin calculated on the basis of the oxygen carrying capacity (Van Slyke method). Blood specimens were collected every two hours from 8 a.m. until 6 p.m. It will be noted that although in a few instances the variations ranged within very narrow limits, as much as 26 per cent variation was noted in two cases. In four cases the variation ranged between 15 and 20 per cent, and in six cases between 10 and 15 per cent.

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LABORATORY METHODS

CONCERNING THE CROSS-FIXATION OF SYPHILITIC ANTIGEN BY TUBERCULOUS SERA*

By Robert A. Kilduffe, A.B., A.M. M.D., Los Angeles, Calif.

IN the maze of literature encompassing the complement-fixation test for syphilis, tuberculosis is not infrequently mentioned as among the conditions giving nonspecific fixation with syphilitic antigens.

It is certainly true that the earlier literature abounds in statistics of numerous nonsyphilitic conditions said to have given such false positive reactions, and it is equally true that the majority were due to errors or inherent faults of technic. It is also true, however, that positive complement-fixation reactions have occurred with tuberculous sera and syphilitic antigens under careful technical conditions and when the presence of coincident syphilitic infection was not easily demonstrable.

Such reactions have several possible explanations: (a) That they are nonspecific and due, in some way, to an alteration of the lipoid metabolism by the Bacillus tuberculosis; (b) That they have some relation to the high lipoid content of the bacillus; (c) That they are specific and indicate a coincident syphilitic infection.

In support of the last view it may be recalled that the incidence of syphilis is fairly high in the tuberculous; that there are some adherents of the view that syphilis may be looked upon as a predisposing cause of tuberculosis, in that it lessens the general resistance to disease in general and to this disease in particular; and, finally, that whenever comparative investigations have been made, the percentage of tuberculous patients giving a positive complement-fixation test for syphilis has been quite comparable to the incidence of syphilis in a similar stratum of the population.

It is not the present purpose to discuss, however, these broad phases of the question, but merely to present the results of a series of tests upon cases of tuberculosis.

The sera examined were secured, through the courtesy of Dr. Joseph Shilene, to whom acknowledgement is due, from one hundred and four cases of tuberculosis (pulmonary) in varying stages of activity.

The complement-fixation tests were all made according to the quantitative method described by Kolmer[1] using as an antigen the extract also described by the same worker.[2] All sera were tested within forty-eight hours of their receipt.

*From the Laboratories of the Pittsburgh Hospital.
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A clean-cut negative reaction was obtained with ninety-four of the sera, ten being positive in varying degree.

In two of the cases reacting positively a specific history was obtainable, one having had a chancre eight, and the other thirty-five years before.

A third case presented a typical secondary eruption clearing up under antiluetic treatment, and a fourth, while denying syphilis, presented a general adenopathy and was of admitted irregular habits.

Two cases were lost and could not be followed up, and in four there was no history, and clinical evidence of syphilis could not be demonstrated.

The denial of syphilitic infection must always be taken with the proverbial grain of salt and the recent report of McConnell[3] serves to emphasize the fact that the lack of *clinically* demonstrable signs of syphilis cannot be taken as absolute evidence of the absence of the disease in the presence of a positive complement-fixation reaction.

While a negative complement-fixation reaction can never be allowed to overshadow the presence of clinical signs of the disease the converse is not true. A positive reaction cannot be summarily dismissed because of lack of clinical evidence, but necessitates an exhaustive search for signs of syphilis which, however, may only be demonstrable at autopsy.

CONCLUSIONS

The data resulting from the small series herewith presented indicates that there is little support for the assumption that tuberculosis per se can give rise to a false positive complement-fixation reaction with syphilitic antigens, and such positive reactions, when obtained in tuberculosis, necessitate an exhaustive examination for evidence of coincident syphilis.

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SHIPPING PATIENTS' SERUMS LONG DISTANCES IN THE TROPICS FOR THE WASSERMANN TEST*

By E. M. Knights, Managua, Nicaragua

As far as we have been able to determine, the following method for shipping serums for the Wassermann work in the tropics, has never been described. We were driven to its use in an effort to make the Wassermann test available for those outlying districts of Nicaragua which are several days' journey from the central laboratory at Managua.

*From Laboratorio de Higiene, Departmento de Uncinariases, Managua, Nicaragua. E. M. Knights, director.

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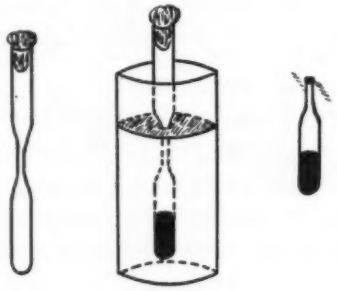
A year's trial has proved it to be a success, and we have at hand a letter from Dr. L. B. Bates, at Ancon, Panama Canal Zone, commenting on the excellent condition in which serums arrived at his laboratory, after a ten days' journey in the tropics. During the journey the serums were at "mail pouch temperature."

The method simply makes use of the fact that during the process of inactivating serums (that is, placing them in the water-bath for 30 minutes at 55° C.) practically all contaminating bacteria are killed. If, therefore, we remove the clear serum from the patient's clotted blood, and place it in long-necked, sterile ampules (Fig. 1) we can inactivate the serum, and then seal the ampule in a flame, thus eliminating all chance of contamination.

Since we use only inactivated serums in our work, these serums are already prepared for the test when they arrive at the central laboratory. In order that proper care shall be used in inactivating these serums in the branch laboratories of the outlying districts, we allow only technicians who have received our personal training to do the work.



1. 2. 3.

Fig. 1.—Cotton-plugged sterile ampule.
Fig. 2.—Ampule in the bath, showing the depth to which it should be submerged.
Fig. 3.—Ampule sealed for shipment.

1. Draw the blood with a sterile syringe.

2. Place the blood in a sterile test tube, slant the tube, and allow the blood to clot.

3. Break up the clot with a sterile glass rod.

4. If centrifuge is not available, stand the tube upright over night (in ice box if one is available) to allow the cells and clot to settle and facilitate the removal of the clear serum.

5. With a sterile pipette, remove the clear serum, using care not to disturb the red cells, and place the serum in a sterile ampule (Fig. 1) for inactivating.

6. The ampule should then be placed in the water-bath which has been regulated at 55° C. Keep the ampule always in an upright position, and do not let it rest on the bottom of the bath. The water in the bath should come well up over the constriction in the neck of the ampule, thus insuring more complete sterilization of the inner surface of the ampule. (Fig. 2.)

There will be a tendency for the ampule to float, therefore it should be

fastened to something that will hold it submerged to the proper depth in an upright position.

7. Keep the temperature of the bath *exactly 55° C. for 30 minutes.*

8. Remove the ampule as soon as the half hour is up. Allow it to cool down to room temperature, keeping it always in an upright position.

9. Seal the constriction in a flame, using care not to heat the serum.

AN IMPROVED ARRANGEMENT OF A SIGNAL MAGNET*

By J. A. Waddell, Charlottesville, Va.

THERE are available in every laboratory devices, mostly electrical, for recording on the tracing the instant the electric current is closed or opened. The usual apparatus and the conventional arrangement of it is not satisfactory for marking the point of delivery of a stimulus to rhythm-

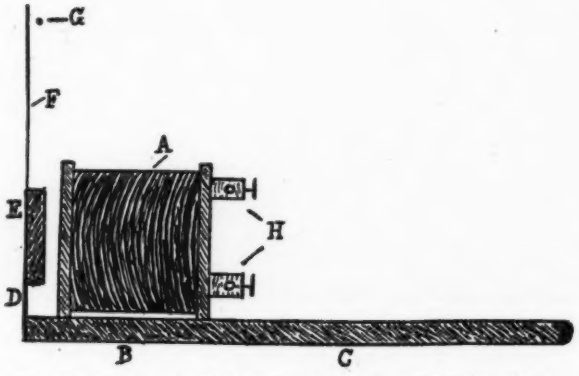


Fig. 1.—Diagram of signal. *A*, Electro-magnet; *B*, supporting frame; *C*, rod for clamping to stand; *D*, armature spring; *E*, armature; *F*, tempered brass wire; *G*, cross section of recording lever, represented by a dot; *H*, binding posts.

ically contracting smooth muscle or in determining the refractory period of the myocardium. Rapid determinations are not practical and exact ones are very tedious. In the first place, the apparatus must be so set up as to attain exact alignment of the writing points. This alignment, standard only for the initial position of the levers, must be maintained throughout the course of the experiment, if accuracy is to be secured. This is practically impossible since a slight variation in the pressure of one or the other of the writing points will alter their relative positions. In the second place, even were it possible to maintain exact alignment, one cannot read directly on a sweeping arc the point which corresponds with the mark made by the signal, but must stop the drum, draw base lines, erect verticals, and describe

*From the Pharmacological Laboratory of the University of Virginia.
Received for publication, June 25, 1923.

arcs—or else apply a scale prepared for each tracing by drawing the above analysing lines.

In recent investigations of the effect of certain drugs on the refractory period of heart muscle and the response of smooth muscle to stimuli, I found it necessary to make exact readings of the instant of application of the stimuli without interrupting the tracing. The following was devised to meet the requirements: It is best shown by reference to a diagram.

The apparatus is set up so as to meet the following in respect to the recording lever: (1) The magnet (A) is placed at right angles; and the wire (F), pointing vertically upward, just within and near the writing extremity (not in direct contact). The magnet is connected in circuit with the primary of the induction coil, in the usual manner. On makng circuit, the wire (F) is attracted toward the magnet, this movement bringing it

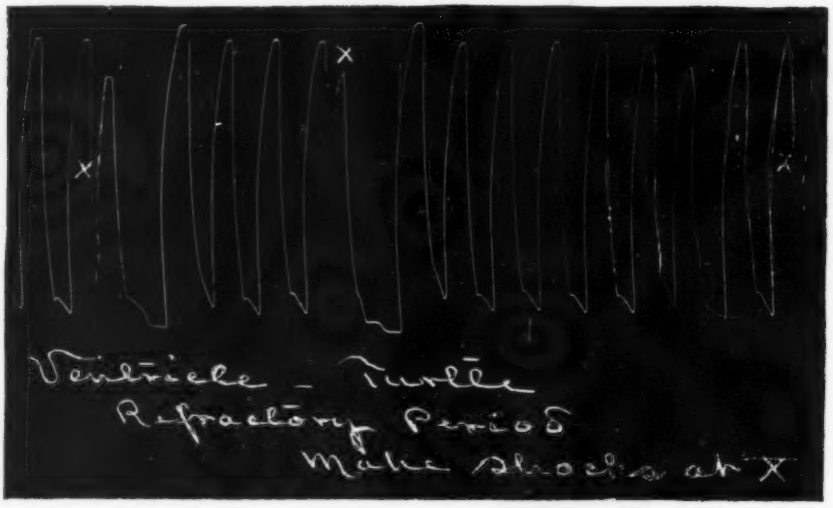


Fig. 2.—Tracing, refractory period, showing at *X* the points at which make stimuli were delivered. The break stimuli were short circuited.

into contact with the recording lever, thus pulling the writing point away from the drum so as to leave a blank space in the tracing. The opposite with resumption of the record takes place on breaking the circuit.

The apparatus may be easily constructed from materials usually found in a laboratory work-shop; for instance, from the ordinary form of signal magnet, provided it has a sufficiently large coil, or from an electric call bell. Care should be exercised to have the wire (F) perfectly smooth and accurately vertical, so that it will not interfere with the excursions of the writing point and alter the characteristics of the tracing.

This arrangement of signal magnet has the following advantages:

1. The apparatus may be quickly set up.
2. Delicate adjustments do not have to be made.
3. The exact point of delivery of a stimulus is seen at a glance.
4. Effects of progressively increasing doses on the response to electrical stimulation may be determined without interrupting the experiment.

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EDITORIALS

Tuberculosis "Cures"

TOO frequently in the lay press we see announcements of "cures" for tuberculosis. It is interesting that those prominently paraded in recent years have mostly been variations of "tuberculins," first announced by Koch as "cures" for this disease. We continue to get reports in the German medical literature of the good results obtained by the inoculation of the living attenuated tubercle bacilli obtained from the turtle by Friedmann. This treatment was tried out in the United States in 1912-13, following a visit to this country by Friedmann, and an unprecedented booming by the lay press, and it was definitely determined by competent medical men that this treatment did not cure. For several years, in Europe, the newspapers have been playing up the Spahlinger "cure." A campaign is at present under way in the London newspapers to raise money to assist Spahlinger. The theme again is that of tuberculin, masquerading under the name of antigen, the tubercle bacilli, according to an article in the Lancet, having been "harried" to induce them to give off toxins, before being killed by diffused daylight. In addition Spahlinger claims to have isolated twenty different toxins—no scientific investigator having yet produced one—with which horses are immunized and sera obtained for the treatment of febrile

cases. No carefully controlled scientific studies on cases treated with these remedies have yet appeared, although prominent physicians have frequently been referred to as advocates of the method. We find in the literature that Donna employed tuberculin, in which the bacilli had been killed by sunlight, and the Henrys developed a tuberculin in which the cultures had been killed by ultraviolet rays. The results from these tuberculins were disappointing.

Victims of tuberculosis are now clamoring for the Dreyer tuberculins, which we understand are prepared from tubercle bacilli, from which the wax and fat have been removed. Reports from London hospitals of a few cases treated with this new variety of tuberculin are published. The reports are too new and too few to inspire confidence.

For several years the Much partial antigens have been having some trial in this country. These variations of tuberculin are in part fat free, but no extraordinary percentage of cures has, as far as we know, resulted from their use.

It is claimed for all the products above named that no serious reactions follow their employment, and that should reactions occur, it can be predicted that no harm to the patient will follow.

Everyone who has employed the Koch tuberculins has been struck by the excellent results obtained in many patients following the production of a reaction. At the same time these same physicians have seen serious illness and death quickly follow the reactions produced by Koch's tuberculins.

There can be no doubt that many of the excellent results claimed by the originators of these new tuberculins, or antigens, are in patients who are not truly victims of tuberculosis. The difficulty in making a correct diagnosis in patients with pulmonary complaints, in whom tubercle bacilli are not present in the sputum, is well known to all good clinicians.

Physicians in general will remain thoroughly sceptical of all claims for tuberculosis "cures," especially those which are merely variations of the original tuberculins. Tuberculin still holds a place in the treatment of certain forms of tuberculosis, especially in the so-called "surgical" types. It is a remedy which may be very harmful in some cases of pulmonary tuberculosis. Such variations of tuberculin as those of Spahlinger, Much and Dreyer should not be proclaimed as "cures" to the unfortunate victims of tuberculosis.

—G. B. W.

Relation of the Clinical Laboratory to the General Practitioner

JUST by way of introduction let me repeat something that has a very direct bearing on my topic. I take it from the writings of Bill Nye. He says, "I have just been sent to the hospital for twenty days. My physician did it. He did it with an analysis. Anybody who amounts to anything nowadays gets analyzed. Sometimes you find casts, sometimes you find maple sugar and sometimes you find acids, oxides, paints, oils, varnish, white lead, borax, albumin, lime, hair and cement. In these cases the patient should

be placed upon a strict diet, or he will in the course of his life become a corpse." Later on he says: "Sunday, 3 p. m. An analysis today shows more casts, fibrin, gelatine and some zinc and copper. The chemist also discovers that in 1853 I fell from an apple tree and tore my panties in two places. He also says I shall be unhappy with my third wife. She will be unhappy also."

From these quotations and from what I shall say you will be able, by making comparisons, to see how great changes have come into the laboratories—at least into some of them, and at least as far as the panties are concerned.

A clinical laboratory is not a diagnostic institution. By that is meant that it is not an instutition in which the main object is to make diagnoses by means of specimens.

It is an institution—a work shop—in which data of clinical value are sought and given to physicians to whom, when they are added to his clinical history, they have a definite meaning with respect to his interpretation of a patient's ills. By means of such data the physician is more easily able, (or more quickly able) to arrive at a diagnosis, more accurately to estimate a prognosis, and more confidently to institute logical therapeusis.

The clinical laboratory has developed as a result of the enormous expansion and rapid growth of the medical sciences, both of which have made it well nigh impossible for a man, no matter how gifted, to supply all the necessary perspective and erudition that goes with medical practice. The practice of medicine has tended to break up into specialties, first into internal medicine and surgery, later into various subdivisions of these major specialties. From both medicine and surgery the laboratory has grown until it, too, has become a specialty. And now, even the laboratory itself has its specialties,—serology, bacteriology, chemistry, radiology and the like.

In but few instances have clinical laboratories risen to the place they ought to occupy, for the reason that the need of them has grown at such surpassing speed, that the demand for heads has exceeded the supply. Thus it has come to pass that they have been organized chiefly to turn out reports, so to speak. Groups of physicians, hospitals, here and there, clinics everywhere have demanded technicians, not clinical pathologists, and have been willing to pay but technicians' wages up until the most recent times. And they have got just what they deserved—technicians' services. They have *not* got what they ought to have—very special skill and knowledge based upon training and experience. And therefore, the laboratory has become too frequently only an adjunct of the group, of the hospital, of the clinic, instead of being an essentially integral part of each, upon a consultation basis. In the proper clinical laboratory the chief should be chosen partly for his skill, partly for his experience and judgment. To him the physician should come with problems to discuss, to get perspective, to get advice and information gleaned from fields which the practitioner cannot even be expected to know; for how can the busy doctor find time to read the various more specialized journals of medical chemistry, bacteriology, immunology and

the like, when he has scarcely time, if he have even so much, to read his own local state journal and his national medical journal?

Any experienced laboratory man can train technicians to do the major part of the routine work, but whom shall the doctor trust for interpretations? And it is the interpretation that counts for most. Will a technician be trusted to interpret gastrointestinal x-ray work? Yet it is quite true that some technicians can make better plates than many a radiologist. The true radiologist is not a maker of picture books. The tissue pathologist is not a producer of pretty slides.

It has been said that the main object of a clinical laboratory is not to make diagnoses, but to discover facts. Let it be added that it should be an important duty of a clinical laboratory to help the physician, when need be, fit the facts together with the other clinical facts he has discovered.

One outstanding reason why a laboratory should not make diagnoses is that so much of its work, or the results of its work, is negative. Take for instance the every day problem of examination of sputum for tubercle bacilli. If the result of a study of smears for tubercle bacilli is a "negative" report, does that mean that the patient in whom the specimen originated is not affected with pulmonary tuberculosis? Of course not! But such a report taken together with other clinical data which the doctor of the patient has collected may mean a great deal. It will mean still more to the doctor if, in addition to the negative report, the pathologist adds other remarks relating to the specimen. If a specimen of sputum is worth sending to a laboratory it is worth study, and should be described and then investigated for tubercle bacilli, and, during the examination for these organisms, other things should be noted *and made a part of the report—provided the speci men has been decently* (this term is used advisedly) *obtained and properly preserved.* For after all tuberculosis is not the only pulmonary disease which is of interest to us, and by attending to minor details the clinician can help the pathologist to help him. Most specimens of sputum submitted for examination are suitable only for search for acid fast bacilli. Many of them are not sputum but the secretions of the upper respiratory tract, obtained by hawking, not by coughing.

Much the same comments might be made upon the study of smears for gonococci, B. diphtheriae and the like. In other words it is up to the clinician to make the applications whether it be silver nitrate or facts. But if the two can get together, the clinician with his data and the pathologist with his, both with a comprehensive knowledge of correlated but different fields, the patient may benefit.

To revert for just a moment to the tubercle bacilli and the gonococci: In either case a smear called positive, is not *positive*,—it is *probable.* Animal inoculation in the one case, and cultural experiments in the other give proof, but such things take time and are expensive and are usually unnecessary. The clinician wants speedy help, as speedy as reasonable accuracy will permit. Rapid laboratory evidence plus the clinical history is usually all that is required. Therefore, the report should read something like this: "Microscopically, typical gonococci are present in the smears."

There are zones in laboratory work in which discovered facts may justify diagnosis. One of these is that which we may call the tissue zone; another the radiologic. In the one, the pathologist may be able to recognize accurately certain types of tissue change as pathognomonic; also he may be able to discriminate certain types of infectious processes. In the other, the radiologist is able to make very exact diagnoses in cases of fractures and dislocations, and in the localizations of foreign bodies, but in very many cases he must take carefully into account the clinical aspects of the case and the processes that modify the tissues.

A pathologist who makes snap shot diagnoses of tissue fragments excised for diagnostic purposes takes long chances unless he knows the surgeon. At the edge of malignant growths there is apt to be a region of hyperplasia, and it may be that this region has furnished the specimen he is asked to describe. In this field of pathologic histology the ordinary recent graduate has no business to wander unguided. Not until after a very wide experience can he be safe in deciding the boundary disputes between inflammatory hyperplasia and sarcoma; between epithelial overgrowths and epithelioma; between adenoma and carcinoma; between lymphomas and lymphosarcomas; and to evaluate the irregularities in that no man's land of precancerous states.

A somewhat analogous situation exists in the serologic zone. How shall a technician evaluate a Wassermann test? Does a so-called "Two plus Wassermann" mean lues? What does a "doubtful" mean? It takes both the clinician and the laboratory man to discriminate in such cases. Moreover, with the demand for some sort of quantitative Wassermann tests it takes a more proficient technician than most of us have been employing to do the very accurate work demanded. It has been said that the quantitative Wassermann test is all "bunk." It probably is in very numerous laboratories, and yet it is possible with a minimum of care to estimate the variations that exist in cases of mild luetic infections, and those that develop in the course, and as a result of, treatment. When, after six years of intensive work Kolmer has come to rely upon a quantitative test, there is no one who should dispute his conclusions, unless he has made at least as comprehensive studies. Anyone who wishes can prove for himself the fact that the quantitative test is accurate.

Kolmer, after six years' intensive work on the Wassermann reaction says: (Am. Jour. Syph., January, 1922.) "Probably the majority of serologists follow the principles of a quantitative method gradually evolved from the work of Wassermann and Citron, that is, Wassermann's one tube test with Citron's method of reading the reactions as strongly positive, weakly positive, doubtful and negative. Contrary to general opinion this method is only partially quantitative. For example, a serum may contain four units of syphilis antibody and give a + + + +, or strongly positive reaction; a second serum may contain ten units of antibody, but cannot give a result stronger than the same + + + + reaction. In other words, a certain amount of antibody is required to give a + + + + reaction in any given test conducted with a fixed amount of serum as 0.1 or 0.2 c.c.; if the serum contains more

than this amount of antibody, it is not shown in the reaction. Only when the amount is reduced below this amount may the reactions gradually become weaker as + + +, + +, +, and negative. In practice, the serum of a patient yielding a + + + + or strongly positive reaction before treatment is instituted may continue to react in this manner for an indefinite period until the amount of antibody is reduced to the + + + level; *this may give the physician reason for suspecting that treatment is having no effect at all upon the strength of the reaction,* whereas more accurate quantitative methods, employing graded doses of serum or complement may show that the amount of antibody is actually being reduced. A one tube test (that is, a front or antigen tube and a rear tube for serum control) is satisfactory for determining whether or not a serum or cerebrospinal fluid contains syphilis antibody, but yields only a rough gauge of the amount of antibody. For this reason, I prefer to call it a qualitative rather than a quantitative test for the reasons given above. This test is perfectly satisfactory for determining whether or not a fluid yields a positive or negative reaction, but as a measure of the degree of positiveness it is unsatisfactory and should give way to more accurate quantitative tests. *An accurate quantitative complement-fixation test for syphilis is essential to show the severity of infection and the effect of specific treatment.*

In the fluid field—or shall I say swamp?—of urinalysis there are grave dangers. Imagine making a diagnosis for a man by the mere urinalysis.

In one of the Falstaff plays of Shakespeare, there is told the story of the analysis of a specimen of urine. It seems that Falstaff, fearing for himself and having read in the newspapers that upon a urinalysis a complete diagnosis and prognosis could be effected, sent a specimen of his urine to a chemist who in his turn submitted the following report: "The urine itself is a very healthy urine, but the person who had the passing thereof may have more diseases than I know of." That report proves that Falstaff didn't send the specimen to the chemist who had written the advertisement.

But after all despite the very many things that might be said of the chemist's report, I use it only for the purpose of indicating that Shakespeare knew, and that was a long time ago, that a normal urinalysis does not necessarily indicate health, and also to call attention to the corollary that an abnormal urine does not necessarily indicate disease.

There is not a single item looked for in a routine urinalysis which of itself means disease either of the kidney or of the body. What they do indicate is the trail that leads to disease. The physician who is to be guide upon the trails must know more about his patient than the mere urine analytic features. He must know how his patient lives. To find the trail and head his patient back to normal he must know at least the main facts of renal physiology, which is to say, the physicochemical landmarks of the living organism. In the early stages of variation from the normal it is simple to advise a case successfully, but how few do it!

Shakespeare's chemist knew that Falstaff suspected something wrong, but he found nothing. Indirectly he suggested that what Falstaff needed

was a thorough physical examination to discover why he should be worried and still have a normal urine.

In the clinical study of a renal disease attention in the past has been centered largely upon the urine, and conclusions as to the function and pathology of the kidneys have been based too generally upon urinalyses. How unstable such procedures are can best be seen when urinary findings and postmortem studies are correlated.

J. R. Stark working in my laboratory in the Cincinnati General Hospital made a series of observations on the correlation of clinical and anatomic findings, with the result that the clinical and anatomic diagnoses agreed in but 36 per cent. In this group there were but 14 cases which showed a typical urinalysis. Stark's series comprised 600 cases, in 560 of which there were some renal lesion, and of this number there were only 63 cases in which there was any attempt on the part of the clinician, judging from the clinical diagnoses, to attach the urinary findings to a renal lesion—which indicates that the clinician himself lays but small stress upon urinalysis alone as a diagnostic measure. (Perhaps he depends too much upon the laboratory).

Certain conditions have been shown to be requisite for the secretion of a normal urine. In the first place, there must be at least 18 to 20 per cent of normal kidney weight. In other words, an animal may have lost from 75 to 80 per cent of his kidney substance and still preserve an adequate function provided the surviving tissue is normal. In the second place, the vascular system must be sufficient, for naturally enough unless an adequate amount of blood can reach the kidneys, and unless the renal vessels have a normal permeability the system will be lacking. In the third place, there must be a sufficient supply of free water, for otherwise the excess of salts cannot be carried out of the body in normal amounts and will accumulate; and in the fourth place, there must be a free outlet from the body, for otherwise the fluid will be dammed back in the urinary system and will impair the renal secretory activities.

The facts are that the glomerulus secretes water and salts, and that the tubular system resorbs salts and excretes nitrogenous bodies. That being the case one should be able to say in case of disease that if there is salt and water retention, the glomerulus is the essentially damaged part of the organ, and that if nitrogen accumulates in the body the disease is essentially tubular. This sharp demarcation of disease can be produced experimentally no doubt, but it rarely occurs in nature, and so the features of renal disease are always mixed. Moreover even fairly sharply delimited disease of the tubules may cause water retention, because swelling of the cells prevents the passage of water from the glomerulus through the tubules, and since that is true there can be no resorption of salts. It is impossible to demarcate the lesions because demarcation does not occur. Inflammatory nephropathies always (speaking generally) affect both glomerulus and tubules.

Another set of factors influences our clinical studies of nephritis, and these have to do with the distribution of renal lesions. In some cases the changes affect the whole organ—they are diffuse. In others they affect only

parts of the organ—they are focal—"spotty." What does this mean? This, that if one part of the kidney is normal that part produces a normal urine. If one part is abnormal it produces an abnormal urine. The two urines mix in the pelvis and in the bladder and the result is, of course, composite. That is one reason why urinalysis alone tells so little in many cases. It may even be that if there is sufficient normal tissue left it is able to do the complete secretion and absorption work for the body and so nitrogen bodies do not accumulate in the blood. It is such cases that show a normal or slightly abnormal blood nitrogen, a normal phthalein output, an almost normal urine, yet which are on the ragged edge between health and disability. And what a state of affairs in which one kidney is badly damaged and the other healthy! Here, of course, lack of elimination in one side will be taken care of in the other.

When one takes all these things into consideration he realizes at once that conclusions as to the state of the kidney drawn from studies of the urine alone may be as Dunn says "as brittle as the urinal." A kidney which is 50 to 75 per cent diseased may produce a normal urine, especially when the diseased portions are so damaged that they do not secrete. The normal urine comes from the normal parts of a diseased kidney. Isn't this exactly what happens in a so-called chronic interstitial nephritis?

Recently (Arch. Int. Med., 1923, xxxi, 499) Coffen has reported an intensive study of albuminuria with especial reference to its clinical significance and says: "Albuminuria is not a sure indication of damaged kidneys; for it may appear when these organs are normal, as in orthostatic albuminuria, or it may be absent in a wide spread degeneration of the kidneys as in interstitial nephritis. Furthermore, albuminuria may be excessive from passive congestion of the kidneys, the primary difficulty being in the heart. When albuminuria is excessive and associated with oliguria and apparent uremia, as a late event in arterial hypertension or in circulatory failure, it may cause much apprehension; on the other hand, the absence of albumin in the urine may give a false sense of security." From these and collateral remarks it is readily seen that dependence upon urinary findings alone leads to error.

There must be better methods, therefore, by which renal conditions may be estimated, or determined more accurately, than by those using the urine alone as the object of examination. Such methods appear to be those which make use of blood chemical studies, together with renal test diets. Since the latter are somewhat difficult to carry out without expert dietetic assistance, and are therefore most suitable for patients who are hospitalized, and since practically all that is needed for blood chemical studies is a very moderate state of fasting, this appears to be the general method of choice in deciding, in patients with albuminuria, whether the kidneys are, or are not, at fault. So true is this, Coffen says, particularly of his vascular hypertension group, that in these patients "the chemical examination of the blood was of diagnostic value," and further, "Since making chemical examinations of the blood one has the impression of assurance of the true state of the kidneys which formerly was doubtful."

It may even be that a urine may be normal when a patient is at rest,

but may show wide variation if he works. In such a patient one has a really important clinical fact, for it tells how much functional kidney there is in the patient. In other words physical activity is an excellent functional test, though in infrequent use.

Much the same state of affairs exists in cases which show some apparent disturbance of sugar metabolism. The presence of sugar in the urine may or may not be of great significance. The presence of sugar in the blood, above certain fairly well-defined limits is of great significance. Moreover, the only satisfactory method of estimating the efficacy of treatment is to have accurate blood sugar determinations. In this respect it has the same value as the basal metabolism studies in treated cases of exophthalmic goiter.

These are some of the reasons why the laboratory should give the analytic facts to the physician, and let *him* make the diagnosis. So we may combine analytic studies with synthetic, the object of which is rational treatment and prognosis. These are also some of the reasons why laboratory reports should be made only to physicians. The patient is not able to evaluate the laboratory facts. —*P. G. W.*

COMMUNICATION

September 18, 1923.

The Journal of Laboratory and Clinical Medicine.

Dear Sirs:

In the August number of your journal, page 741, Vol. VIII, 1923, there is an article signed by George F. Spencer, M.D., of a method for estimating pancreatic enzymes. Your staff were misinformed when told that the data from which the above mentioned article was taken, was unpublished. Again it is a modification by myself and Olaf Bergeim of the Gaultier method, quite a different worker than Gautier.

Please believe me that I taught every step of these tests to Dr. Spencer, after I and Dr. Bergeim had worked on these methods for two years, with his full knowledge that the Department of Physiology at Jefferson Medical College was about to publish them. Dr. Bergeim wrote Dr. Spencer, requesting him not to publish any data on our modifications. His paper is a word for word copy of my typewritten memoranda offered Dr. Spencer as aid to learning our methods.

B. B. Vincent Lyon: "Non-surgical Drainage of the Gall Tract," Lea and Febiger, 1923, in a chapter by Dr. Bergeim, gives notice that the original article by myself and Dr. Bergeim, was in the press of the American Journal of Medical Sciences.

Will you give this letter your serious consideration, and have it published in the next number of your journal?

Yours for fair play, and loyalty to medical ethics. I am entirely ignorant of any personal animosity entering into this matter.

Respectfully submitted,

(Signed) C. W. Lueders.

October 25, 1923.

The Journal of Laboratory and Clinical Medicine.

Dear Sirs:

Yours of recent date at hand. In reply will say that I am aware of the fact, and wish it to be known, that it was Drs. Bergeim and Lueders that devised the modified Gaultier's method for the determination of the pancreatic enzymes and not myself, and, in publishing it as a prelude or introduction to the article to follow, I tried to make it plain that they were the originators and not myself, although I did, in a few places, change the method and so informed Dr. Bergeim. I ask no credit for the actual method nor do I wish in any way to minimize the credit due Drs. Bergeim and Lueders.

Dr. Bergeim did ask that I publish no data on the modification but after it had been given out for publication I was to be given the privilege of publishing it either along with or before the publication of the results of the application of the method, a thing which it took me one and a half years to work out.

Dr. Lueders is correct in saying that he knew of no personal animosity between us. There is none. Furthermore, I wish at this time to express my appreciation for the assistance rendered me by Drs. Lueders, Bergeim, Hawk and Shrader in the preparation of reagents, details in technic, and the final presentation of the paper.

Very truly yours,

(Signed) Geo. F. Spencer, M.D.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Duff House Papers**

DUFF HOUSE PAPERS is a collection of contributions from the staff of Duff House. Some have been written expressly for this volume. Although varied subjects are covered, the work naturally falls into two classifications. The first half of the book is devoted to a study of gastrointestinal disease. Considerable prominence is given to the roentgen examination of the alimentary tract as a whole, and of the appendix, stomach, and Meckel's diverticulum in particular. This portion is unusually well illustrated. Most of the remainder of the book is devoted to diabetes and is of particular interest, in that a study of the methods at Duff House shows us points of similarity and of difference in the treatment of this disease as carried out on the two continents. A diabetic manual for the use of patients follows in general the lines that are covered in this country in the books by Joslin and by Wilder, Foley and Ellithorpe. A chapter is included on special home gardening so that the diabetic may have those foods in his own back yard which are particularly desirable. In general, the treatment of the disease as outlined by Spriggs and his associates corresponds quite closely with that followed in this country prior to the advent of insulin. The volume will be of interest particularly to internists and to roentgenologists.

*Diseases of the Gums and Oral Mucous Membranes***

THE author has accomplished the presentation of an exhaustive treatise on the subjects enumerated in the title. He discusses both local disease and those conditions situated in other portions of the body which are held to originate from oral sepsis. The illustrations which consist of drawings and photographs of the local lesions, roentgenograms, and photomicrographs of pathologic issues and infecting bacteria, are unusually good. While the volume would naturally be of value to oral surgeons and to dentists, it will be of particular interest to internists and dermatologists. It may be highly recommended.

*Duff House Papers. Edited by Edmund I. Spriggs, M.D., F.R.C.P., Volume One. Cloth. Price $9.50. Pp. 387. Illustrated by 190 figures, 6 charts and 1 colored plate. London. Oxford Medical Publications. 1923.

**Diseases of the Gums and Oral Mucous Membranes. By Sir Kenneth Goadby, K.B.E., M.R.C.S., L.R.C.P., D.P.H. (Cantab). Lecturer on Bacteriology of the Mouth, Dental Department, University College Hospital; Specialist Medical Referee for Industrial Poisoning, London District (Home office); Late Bacteriologic Specialist, Royal Herbert Hospital, Woolwich; Erasmus Wilson Lecturer, Royal College of Surgeons, 1907; Hunterian Professor, Royal College of Surgeons, 1911. Cloth. Price $14.00. Pp. 383. London. Oxford Medical Publications. 1923.

*The Colloidal State**

THIS book might well have been entitled the "A B C of Colloidal Chemistry." Its author, recognizing the increasing importance of colloidal chemistry in medicine and allied sciences, has written a small volume for those who may wish to acquire a general knowledge of the underlying principles. Dr. Bayliss has eliminated involved mathematical formulas and has so simplified his illustrations that on completion of the book the lay reader may have acquired a sufficiently full understanding of the subjects discussed. In the last two chapters one finds a brief presentation of the relationship of colloidal chemistry to pathologic physiology. We regret that this latter portion of the book is not more detailed.

Heliotherapy†

DR. ROLLIER and his collaborators preach the gospel of sunlight in the treatment of tuberculosis. The treatment of disease by exposure to the direct rays of the sun is as old as the Incas tribes. In no disease, however, has such splendid success been attained as in the treatment of tuberculous infection. Not all forms of tuberculosis respond to the sun bath and in pulmonary tuberculosis, for example, the treatment is applicable only to certain stages of the disease. Best results are obtained in tuberculosis of the bones and joints. Light therapy as practiced in Swiss clinics is beneficial in certain nontuberculous diseases, particularly rickets, Hodgkin's disease, malnutrition and rheumatism. A profusion of illustrative cases with photographs taken before and after treatment tell their own story. The book should be of special interest to those who treat tuberculosis in any form.

Practical Physiology‡

A LABORATORY textbook of physiology whose chief point of difference from the general run of textbooks on this subject lies in the adaptation, to a great extent, of methods similar to those applied in the routine study of disease. In this way the student maintains a closer association between his physiologic laboratory work and the later clinical laboratory work. The book is profusely illustrated and the drawings are unusually clear.

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*The Colloidal State in its Medical and Physiological Aspects. By Sir William Bayliss, F.R.S., M.A., D.Sc., LL.D. Professor of General Physiology in University College, London. Cloth. Pp. 95. Price $2.15. Oxford Medical Publications. Henry Frowde and Hodder and Stroughton. London. 1923.

†Heliotherapy. By A. Rollier, M.D., Medical Director of the Institutions of Heliotherapy, Leysin; Laureate of Academy of Medicine, Paris; Correspondent Member of Societies of Medicine and Pediatrics, Paris. With the Collaboration of A. Rosslet, D.Sc. M.D. Lecturer on Physicotherapy, Geneva University; formerly assistant at Rollier's Institutions, Leysin. H. J. Schmid, M.D., Radiologist at Rollier's Institutions, Leysin. E. Amstad, M.D., Surgeon at Rollier's Institutions, Leysin. With forewords by Sir. John Henry Gauvain, M.A., M.D., M.R.C.S., F.R.S. And Caleb Williams Saleeby, M.D., F.R.S. Cloth. Price $8.00. Pp. 288. London. Oxford Medical Publications. 1923.

‡Practical Physiology. By G. V. Anrep, M.D., D.Sc., Senior Assistant in Physiology, University College, London; and D. T. Harris, M.B., B.S., B.Sc., Assistant Professor of Physiology, University College, London. Introduction by E. H. Starling, C.M.G., M.D., F.R.C.P., F.R.S.; Jodrell Professor of Physiology in University College, London. Cloth. Pp. 212. 197 illustrations. London. J. & A. Churchill, 1923.